The Human B Cell Response to a Multi-Antigen Complex (Bexsero)

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Zusammenfassung

Die menschliche B-Zellantwort auf einen Multi-Antigen-Komplex (Bexsero)

Multi-Antigen-Komplexe wurden erfolgreich in der Impfstoffentwicklung als effizientes Modell genutzt, um eine breite Durchimpfungsrate gegen mehrere Stämme desselben Erregers zu erreichen. Bisher wurde Seropositivität gegenüber Impfstoff-Antigenen nach Impfungen vorwiegend über den Serumspiegel gemessen, häufig bei hohem Titer (z.B. 1:4). Ein großer Nachteil dieser Quantifizierungsmethode ist, dass sowohl die Wirkung der Multi-Antigen-Komplexität auf das Zusammenspiel verschiedener Immun-Akteure in der Keimzentrumsreaktion, als auch der Gesamteffekt auf die Qualität der B-Zellreaktion, nicht nachvollzogen werden können. Jüngste Entwicklungen in den Bestrebungen die Feinheiten der B-Zellreaktion auf Multi-Antigen-Komplexe zu entschlüsseln, deuten allesamt auf eine weitgehend durch die Anwesenheit von multiplen Antigenen angetriebene Veränderung des Verhaltens der B-Zell-Antwort hin, mit der Annahme, dass evolutionäre Vorteile dabei entstehen. Im Folgenden werden die Erkenntnisse über die menschliche B-Zellreaktion auf den Multi-Antigen-Komplex (Bexsero) an drei Impfprobanden (Vax2,Vax3 und Vax4) aufgezeigt. Bexsero ist ein Impfstoff, der aus vier Hauptantigenen (fHbp-GNA2091, NHBA-GNA1030, NadA und OMV (NZ98-254)) besteht, die gegen *Neisseria meningitidis* B entwickelt wurden. Unsere Ergebnisse deuten auf die Entstehung eines (in Hinsicht auf Isotypenverteilung, IgVH- und IgJH-Genexpression, CDR3-Längenverteilung und klonale Selektion) äußerst vielfältigen Immunglobulin (Ig)-Pools als Reaktion auf Bexsero hin, mit nachgewiesen einzigartigen Ig-Gen Selektionsmustern in allen drei Impfstoffen.

Unsere Messungen zeigen ebenfalls Ig-Pools auf, die eine Reihe von Spezifitäten (Bexsero-reaktive Antikörper (ausschließlich in Vax3), sowie polyreaktive Antikörper (in Vax2, Vax3 und Vax4)) und Affinitäten (stark bindende, mäßig bindende, schwach bindende und nicht reaktive Antikörper) aufweisen. Wir konnten keine eindeutige Korrelation zwischen spezifischen Ig-Genmerkmalen und Ig-Reaktivitätseigenschaften erkennen, was die bestehende Schwierigkeit die Ig-Reaktivität aus Ig-Gensequenzen vorherzusagen weiterhin bestätigt; obwohl Igs von allen Probanden verschiedenste Affinitäten innerhalb und zwischen Clustern und unter Nicht-Clustern zu Bexsero aufwiesen, was mögliche Vorteile im Aufbau eines breiten Impfschutzes mit sich bringt. Es wurde ebenfalls Ig-Gen-Merkmale und Antigen-Reaktivitätseigenschaften von Antikörpern aufgezeigt, die gegen NHBA (22 Antikörper), fHbp (2 Antikörper) und NadA (2 Antikörper) erzeugt wurden. Diese Immunoglobuline zeigten in Untersuchungen schwache Bindungsaffinitäten gegenüber

endogen exprimierte Antigene auf *Neisseria meningitidis mc58*, möglicherweise aufgrund eines ungeordneten N-terminalen Endes von NHBA, was eine weitere Charakterisierung verhinderte; wobei dieser Antikörper für strukturelle Untersuchungen von NHBA relevant werden könnte. Die Anreicherung von stark mutierten polyreaktiven Antikörpern während der B-Zellreaktion auf Bexsero, was möglicherweise einen Hinweis auf die Reaktion auf vielfache/vielfältige Antigen-Komplexe darstellt, um eine breite Impfschutzdeckung mittels eines relativ kleinen Antikörperpools zu erreichen. Die angeführten Ergebnisse weisen ebenfalls auf einen unterschiedlichen Grad der Immunselektivität auf die verschiedenen Bexsero-Antigene hin, was auf eine Antigen-Immundominanz hindeutet und Auswirkungen auf die B-Zellreaktionsqualität auf Bexsero mit sich tragen kann. Wir führen danach anschließend an die zweiten Primärimpfung mit Bexsero beobachtete Hinweise auf "Epitop-Maskierung" an, mit Implikationen für die B-Zellreaktionsqualität auf Bexsero, die wichtige Diskussionspunkte über Booster- und Sekundärimpfungen aufwerfen. Schließlich bekräftigen die Einzelzellanalysen von Plasmablasten- und Serenmessungen nach Bexsero-Impfungen die von Novartis empfohlene Impfdosierung, sowie das Impfschema (um eine breite Abdeckung zu erreichen) von zwei verabreichten Dosen pro Erwachsenen in einem Monatsintervall, und legt ein Zusammenspiel zweier immunologischer Phänomene nahe (Immunselektivität/Immundominanz, und Epitop-Maskierung), welches für die Entscheidungsbestimmung verantwortlich ist, die in klinischen Studien sonst über Seropositivitätsmessungen getroffen wird. Zusammenfassend stellt die Immunselektivität/Immundominanz sicher, dass sich die B-Zell-Antwort nach der ersten Primärimpfung bei Kontakt mit Multi-Antigen-Komplexen unterschiedlicher Antigenizität auf ausgewählte Antigene konzentriert, während Epitop-Maskierung und phagozytische Entfernung von Antikörper:Antigenkomplexe die Reaktion von B-Zellen auf verbleibende Antigene in nachfolgenden Impfungen richtet, um die Erzeugung von Antikörpern gegen alle bzw. die meisten Antigene eines Multi-Antigen-Komplexes zu gewährleisten.

Infolgedessen eröffnet sich mit diesen Ergebnissen ein möglicher Weg mit Hilfe eines kontrollierbaren Systems von 4-Antigenen in Bexsero die B-Zellantwort auf Multi-Antigen-Komplexe beim Menschen besser zu verstehen (Pathogeninfektionen, sowie Impfungen) und betont die Notwendigkeit ein tieferes Verständnis über die zellulären und humoralen Einzelheiten der Immunantwort auf Impfstoffantigene während klinischer Impfstoffversuche zu erlangen.

Synopsis

Multi-antigen complexes have been exploited in vaccinology as an efficient model, to achieve broad vaccine coverage against multiple strains of the same pathogen. To date, seropositivity to vaccine antigens post-vaccination, have been measured primarily at the serum level often at high titers (eg. 1:4). A major pitfall of this quantification strategy is that, it ignores the effect of multi-antigen complexity on the interplay between various immune players in the germinal center reaction and the overall effect on the quality of the B cell response. Recent developments to unravel the fine details of the B cell response to multi-antigen complexes collectively demonstrate or propose altered behavior in the B cell response largely driven by the presence of multiple antigens, albeit evolutionarily advantageous. Here, the findings on the human B cell response to a multi-antigen complex (Bexsero) in three vaccinees (Vax2, Vax3 and Vax4) are shown. Bexsero is a vaccine comprising of four main antigens (fHbp-GNA2091, NHBA-GNA1030, NadA and OMV (NZ98-254)) developed against *Neisseria meningitidis* B. An immensely diverse (isotype distribution, *IgVH* and *IgJH* gene usage, CDR3 length distribution and clonal selection) immunoglobulin (Ig) population generated in response to Bexsero with unique Ig gene selection patterns in all three vaccinees was observed. The data also pointed to Ig populations that exhibit a range of specificities {Bexsero-specific-reactive Igs (Vax3 Only) and polyreactive Igs (Vax2, Vax3 and Vax4)} and affinities (highly binding Igs, moderately binding Igs, weakly binding Igs and unreactive Igs). No unique correlation between specific Ig gene features and Ig reactivity properties was observed, supporting existing difficulty in predicting Ig reactivity from Ig gene sequences, albeit Igs from all vaccinees collectively exhibit varied affinities within cluster Igs, between cluster Igs and amongst non-clusters to Bexsero, with potential advantages for broad protection. Ig gene features and antigen-reactivity properties of Igs generated against NHBA (22 antibodies), fHbp (2 antibodies) and NadA (2 antibodies) are also shown. These Igs exhibited weak binding affinities when tested on endogenously expressed antigens on *Neisseria meningitidis* mc58, potentially due to disordered N-terminal of NHBA, hence ending their further characterization, although they may be relevant in structural studies of recombinant NHBA. Enrichment of highly mutated polyreactive Igs, which may be a hallmark of the human B cell response to multiple/complex antigens to achieve broad coverage with a relatively small population of antibodies, was also observed. The data also pointed to varying degrees of immunoselectivity to the different Bexsero antigens, suggesting antigen immunodominance, with implications on the quality of the B cell response to

Bexsero. Evidence of “epitope masking”, observed post-second primary vaccination with Bexsero may also have implications on the quality of the B cell response to Bexsero and raises important discussions on booster/secondary vaccinations. Finally, the single B cell analysis of plasmablast and sera analysis post-Bexsero vaccination buttresses the Novartis vaccination dosage and vaccination scheme (to achieve broad coverage) of two doses per adult administered at one-month interval and suggests the interplay between two immunological phenomena (immunoselectivity/immunodominance and epitope masking) as responsible for defining decisions made otherwise with seropositivity data during vaccine clinical trials. In short, it appears immunoselectivity/immunodominance ensures that upon exposure to multiple antigens with varied antigenicity, the B cell response focuses on select antigen(s) post-first primary vaccination with a multi-antigen complex whereas “epitope masking and phagocytic clearance of antibody:antigen complexes” refocuses the B cell response to remaining antigen(s) in subsequent vaccination steps to ensure the generation of antibodies against all/most antigens of a multi-antigen complex.

In conclusion, with a controllable system of 4-antigens in Bexsero, the data opens a potential window to understanding the B cell response to multi-antigen complexes (pathogen infections or vaccination) in humans and evinces the need for expansive understanding of the fine cellular and humoral details of the immune response to vaccine antigens during vaccine clinical trials.

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To the Adiebah family of Tarkwa, Ghana, this is for you.

To my parents, Yalley family of Koforidua, Ghana and Oppong-Yankey family of Takoradi, Ghana, this is for you.

And finally, to all my friends for their support throughout the years.

"In nothing do men more nearly approach the gods, than in giving health to other men."
— Marcus Tullius Cicero

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Ag.....**Antigen**

Ag-1.....**Antigen-1**

$Al(OH)_3$.....**Aluminum Hydroxide**

AUC.....**Area Under Curve**

BCR.....**B Cell Receptor**

CD4.....**Cluster of Differentiation 4**

CDR3.....**Complementary Determining Region 3**

CD19.....**Cluster of Differentiation 19**

CD27.....**Cluster of Differentiation 27**

CD38.....**Cluster of Differentiation 38**

CD40.....**Cluster of Differentiation 40**

cDNA.....**Complementary DNA**

dsDNA.....**Double Stranded DNA**

D_H.....**Immunoglobulin Heavy Chain Diversity Gene**

ELISA.....**Enzyme Linked Immunosorbent Assay**

FACS.....**Fluorescence Accelerated Cell Sorting**

fHbp.....**Factor H Binding Protein**

fHbp-GNA2091.....**Factor H Binding Protein–Genome Derived Neisseria Antigen 2091**

FWR.....**Framework Region**

GNA2091.....**Genome Derived Neisseria Antigen 2091**

GNA1030.....**Genome Derived Neisseria Antigen 1030**

HIV.....**Human Immunodeficiency Virus**

HEK293 T cells.....**Human embryonic kidney 293 T Cells**

IL4.....**Interleukin 4**

IgG.....**Immunoglobulin G**

IgA.....**Immunoglobulin A**

IgE.....**Immunoglobulin E**

IgD.....**Immunoglobulin D**

IgM.....**Immunoglobulin M**

***IgVH*..**.....**Immunoglobulin Heavy Chain Variable Region**

***IgVK*..**.....**Immunoglobulin Kappa Chain Variable Region**

***IgVλ*..**.....**Immunoglobulin Lambda Chain Variable Region**

IgHγ1 **.....Immunoglobulin Heavy Chain Gamma 1**

Igk1 **.....Immunoglobulin Light Chain Kappa 1**

Igλ1 **.....Immunoglobulin Light Chain Lambda 1**

JH.....Immunoglobulin Heavy Chain Joining Region

JH1.....Immunoglobulin Heavy Chain Joining Region 1

JH2.....Immunoglobulin Heavy Chain Joining Region 2

JH3.....Immunoglobulin Heavy Chain Joining Region 3

JH4.....Immunoglobulin Heavy Chain Joining Region 4

JH5.....Immunoglobulin Heavy Chain Joining Region 5

JH6.....Immunoglobulin Heavy Chain Joining Region 6

LPS.....Lipopolysaccharides

MHCII.....Major Histocompatibility Complex II

NHBA-GNA1030....Neisseria Heparin Binding Antigen–Gen. Derived Neisseria Ag 1030

NHBA.....Neisseria Heparin Binding Antigen

NadA.....Neisseria Adhesin A

NZ98-254.....New Zealand Strain (98 – 254)

OMV.....Outer Membrane Vesicle

PCR.....Polymerase Chain Reaction

PBMCs.....Peripheral Blood Mononuclear Cells

PBS.....Phosphate Buffered Saline

PorA P1.4.....Porin A P1.4

SD.....Standard Deviation

SHM.....Somatic Hypermutation

TCR.....T Cell Receptor

TI.....Thymus Independent

TI-1.....Thymus Independent 1

TH2.....T-Helper 2

Unvax.....Unvaccinated Individual

Vax1.....Bexsero-Vaccinated Individual 1

Vax2.....Bexsero-Vaccinated Individual 2

Vax3.....Bexsero-Vaccinated Individual 3

Vax4.....Bexsero-Vaccinated Individual 4

V_H.....Immunoglobulin Heavy Chain Variable Region

VH1.....Immunoglobulin Heavy Chain Variable Region 1

VH2.....**Immunoglobulin Heavy Chain Variable Region 2**

VH3.....**Immunoglobulin Heavy Chain Variable Region 3**

VH4.....**Immunoglobulin Heavy Chain Variable Region 4**

VH5.....**Immunoglobulin Heavy Chain Variable Region 5**

VH6.....**Immunoglobulin Heavy Chain Variable Region 6**

7-AAD.....**7-Aminoactinomycin D**

Chapter 1: Introduction

Human B Cell Development

B Cells are an important compartment of human adaptive immunity, primarily responsible for antibody production to confer protection against pathogens. They originate and mature from hematopoietic stem cell precursors in the bone marrow [1–3]. These hematopoietic stem cells proceed through several developmental stages marked by the rearrangement and expression of Ig genes (fig. 1).

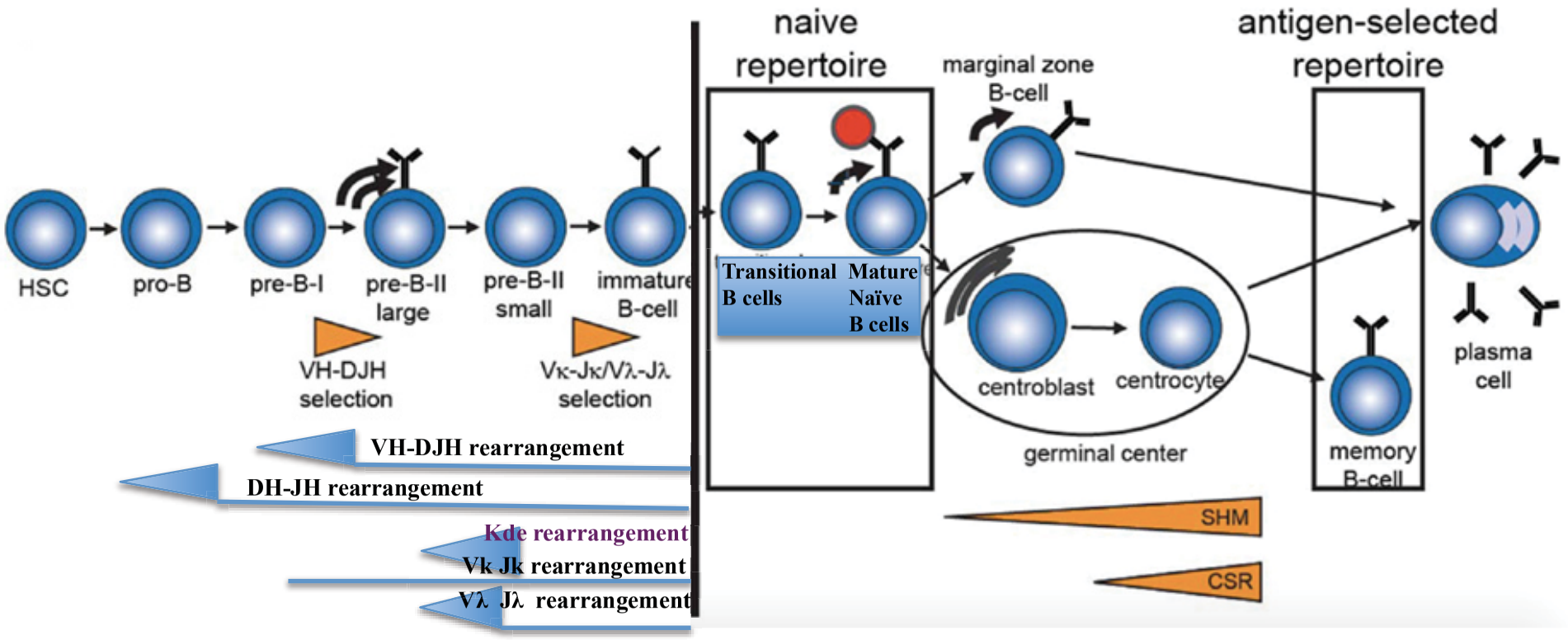


Fig. 1: Schematic depiction of B cell development. Key stages and processes in B cell development [4].

Hematopoietic stem cells destined for the B cell lineage differentiate into a pro-B cell stage which involves the rearrangement of the Ig heavy-chain locus; D_H to J_H join at the early pro-B cell stage, followed by V_H to DJ_H joining at the late pro-B cell stage [5,6]. Intact μ heavy chains are expressed from productive VDJ_H, transitioning late pro-B cells into large pre-B cells. Large pre-B cells combine the μ heavy chains with surrogate light chains to form the pre-B cell receptor [5,6]. The cells undergo several rounds of cell division in which endogenous light-chain

rearrangement begins; V_L combines with J_L to form VJ_L [5–7]. The cells subsequently combine μ heavy chains with assembled light chain genes in immature B cells to express complete IgM molecules [5–7]. The stochastic nature of both Ig V(D)J recombination and heavy-light chain combinations enriches the diversity of the B cell repertoire [5]. Immature B cells subsequently undergo a selection process for self-tolerance and the ability to survive in the peripheral lymphoid tissues [8]. B cells that survive the selection process undergo further differentiation to become mature naive B cells that express both IgD and IgM [7–9]. Mature naïve B cells circulate through the peripheral lymphoid tissues until they encounter and are activated by an antigen [8].

Human B Cell Response to Infection

During infections, adaptive immunity is initiated when the concentration of pathogens exceed the threshold dose of antigen required for an adaptive immune response. Mature naïve B cells in circulation encounter antigen-presenting cells from the site of infection in peripheral lymphoid tissues [8]. Upon encountering their cognate antigen, B cells may either require a secondary activating TH2 cell signal (Thymus-dependent antigen) or not (thymus-independent antigen), to be fully activated. Thymus independent (TI) antigens consists of TI-1 antigens which posses an intrinsic activity that can directly induce B cell division or TI-2 antigens, which comprises mainly of carbohydrate antigens with highly repetitive structures and no intrinsic B cell-stimulating activity [10]. Thymus dependent antigens are mainly protein antigens. Antigens bound to the B cell receptor are processed by phagocytosis and presented as small peptides bound to MHCII on the cell surface [11]. TH2 cells provide the secondary activating signal when they recognize the fitting peptide:MHCII complex on the B cell surface [12]. The binding triggers TH2 cells to synthesize both cell bound and secreted effector molecules like CD40 ligand and IL-4 that synergize in activating the B cell [13,14] Activated B cells undergo several rounds of activation and eventual differentiation into antibody secreting plasma cells [12]. All antibodies secreted via thymic-independent antigen response and the primary phase of thymic-dependent antigen response are predominantly IgM [15]. Several days post-infection, naive B cells and Th2 cells aggregate to form germinal centers [16]. Germinal centers are special B cell follicles where B cells undergo repeated cycles of replication, with somatic hypermutation and selection to generate high affinity antibodies [17]. Germinal centers are also sites of class-switch recombination to generate antibodies with alternate constant regions and consequently, effector functions (IgA, IgG, and IgE). Somatic hypermutation selectively introduces point mutations into the variable region of the B cell receptor at a rate of one mutation per replication cycle [17]. Hypermutated B cells engage with follicular

dendritic cells for antigen and for positive signals from TH2 cells [16]. The cells undergo a selection process, which ensures that higher affinity cells have a higher probability of receiving TH2 cell help and undergoing replication, leaving lower affinity B cells to apoptose [18]. The germinal center reaction referred to as, affinity maturation often terminates after a few rounds but may also last for several weeks [19]. The average affinities of the B cell receptors increase by several orders of magnitude after termination of the germinal center reaction [20]. B cells emerge from the germinal center reaction as plasmablast or memory B cells. Memory B cells may persist indefinitely in circulation without antibody secreting capabilities but help initiate a quick response upon re-exposure to the same antigens [21]. Plasmablast differentiates into short-lived or long-lived antibody-secreting plasma cells. Long-lived plasma cells home to the bone marrow, whereas short-lived plasma cells apoptose post-infection[22–24].

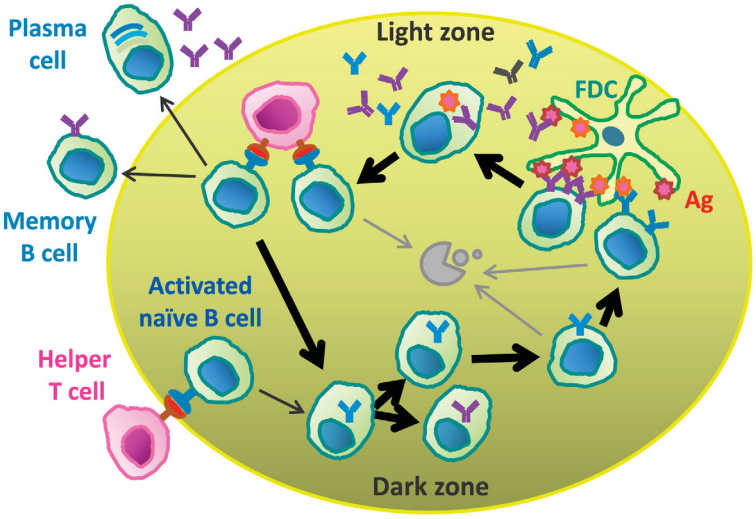


Fig. 2: Schematic depiction of affinity maturation. Key players and processes in the germinal center reaction [25].

Vaccination

Prior to understanding the science of immunology, the immune system was already manipulated to establish protection against infections through vaccination. Advancement in B cell immunology has led to better vaccines. Vaccination is the inoculation of healthy individuals with weakened or attenuated versions of disease-causing pathogens, to provide protection from disease [11]. Vaccines are primarily made from purified protein or carbohydrate antigens obtained from pathogens. Recent carbohydrate vaccines are often conjugated to carrier proteins such as CRM197 – a non-toxic mutant of diphtheria toxin - to enable MHCII presentation of the conjugated carbohydrate – peptides in order to induce germinal center reactions to generate high affinity antibodies. Vaccines with multiple antigen components such as MenACWY-CRM – tetravalent polysaccharide vaccine against meningococcal

serogroups A, C, Y and W - have also been introduced to broaden vaccine coverage [26]. Multi-antigen component vaccines are made either by combining variants of the same antigen from distinct strains of the same pathogen or multiple different antigens from one pathogen strain. Although pathogens vary in their antigenic complexity, the human B cell response to multicomponent vaccines mimics that of natural pathogens. These vaccines therefore provide a controllable system to investigate the human B cell response to multi-antigen complexes. How does the B cell compartment of the human immune system respond when faced with complex antigens? Does the immune system selectively focus on some antigens and/or epitopes over others, upon encounter with a multicomponent antigen? How do multicomponent antigens alter the fine specificity of the antibody response? Are there implications of a trade-off between the breadth (number of antigens and/or number of epitopes covered per antigen) and the efficacy (quality and diversity) of the B – cell response to multi-antigen complexes? Consequently, what are the overall implications for the development of adequate protection against the pathogen of interest? Similar questions were asked by Childs et al, 2015 in which they simulated the germinal center reaction by quantifying the binding affinity of a BCR to an antigen via a genotype-phenotype map, based on a random energy landscape, that combines both local and distant interactions between residues [16]. In the presence of many antigens or epitopes, they found that the germinal center reaction produced an antibody repertoire with both reduced binding affinity and relative breadth [16]. Hicar et al, 2010 also published findings on human HIV-specific antibody repertoires [27]. Albeit, not a direct study of the B cell response to a multi-antigen complex, whole HIV infection presents multiple antigens to the B cell response with antibodies suspected to target virus envelope conformations present only in complex oligomeric structures on virion particles and virus-like particles [27]. They showed that HIV-specific repertoires exhibit a high level of clonality in circulating cells and high levels of somatic hyper mutations, within the antibody gene segments [27]. Kaufmann et al, 2016 have also analyzed the human plasmablast response to *Vibrio cholerae,* which also presents multiple antigens to the human immune system [28]. They showed that high levels of somatic hypermutation and large clonal expansions characterize cholera-induced antibody responses. They also show that anti-cholera antibodies primarily target three main antigens (cholera toxin, LPS and sialidase) suggesting antigen immunoselectivity and immunodominance [28].

With single antigens on the contrary, Kuraoka et al, 2016 have shown in mice that unlike single genetically restricted antigens (e.g haptens), single complex antigens induce diverse patterns of clonal selection in germinal centers [29]. They investigated the population dynamics

of genetically diverse GC responses to two single complex antigens – Bacillus anthracis protective antigen and influenza hemagglutinin – in which B cells competed both intra- and inter-clonally for distinct epitopes [29]. They showed that despite similarity with B cell responses to haptens (single antigens) in which preferential V_H rearrangements among antigen-binding naïve B cells are abundant in early GCs, clonal diversity increased in GC B cells in response to competition by rarer, high affinity clones later [29]. They also observed great variation in inter- and intra-clonal avidity (half of GC B cells did not bind the immunogen), biased V_H gene usage, V(D)J mutation and clonal expansion comparable to antigen-binding cells [29], suggesting potential advantages for broad protection [29]. Moody et al, 2014 have also shown analysis on the human B cell response to an HIV-1 vaccine trial (GSK PRO HIV-002) that used single antigen comprising of clade B envelope (Env) gp120 of clone W6.1D ($gp120_{W6.1D)}$ [30]. They studied the clonal persistence over four immunization rounds and observed evidence of both sequential recruitment of both naïve B cells and re-stimulated memory B cells [30]. They also found evidence of moderate V_H somatic mutations and ultimately concluded that gp120 was strongly immunogenic but induced levels of affinity maturation below that of broadly neutralizing monoclonal antibodies over four immunization rounds [30].

Goal of project: All previous attempts to examine germinal center reactions in the face of antigen complexity, demonstrate altered behavior in the B cell response largely driven by the presence of single or multiple complex antigen(s). Despite some in silico analysis of the human B cell response to multi-antigen complexes and studies on the B cell responses to whole pathogens also comparable to the B cell response to multiple complex antigens, conspicuously missing is any study of the human B cell response to a defined number of multiple complex antigens. The goal of this project is to harness the power of the single B cell cloning technique to decipher the impact of multi-antigen complex vaccination on the fine specificity of the human B cell response and further ascertain what the implications are, for protection against the target pathogen, by characterizing amongst other things, the gene features, reactivity properties, bactericidal activity, target antigens and epitopes of the antibody pool generated.

Vaccine (Bexsero): Bexsero is a multicomponent vaccine designed by Novartis against *Neisseria meningitidis* serogroup B. The vaccine components comprises of four main protein antigens, factor H binding protein (fHbp) conjugated to GNA2091, Neisseria Heparin Binding Antigen (NHBA) fused to GNA1030, Neisseria Adhesin A (NadA) all from *Neisseria meningitidis* mc58 strain and the outer membrane vesicle (OMV) from *Neisseria*

meningitidis NZ98-254 strain, as active ingredients [31]. It is currently one of two vaccines approved for vaccination against *Neisseria meningitidis* serogroup B [32].

NadA: A surface exposed oligomeric protein belonging to the oligomeric coiled-coil Adhesin, involved in binding to epithelial cells [33].

NHBA-GNA1030 fusion protein: Fusion protein product of Neisseria Heparin Binding Antigen and GNA1030, an accessory protein that enhances the immunogenicity of NHBA [31].

fHbp-GNA2091 fusion protein: Fusion protein product of Neisseria Heparin Binding Antigen and GNA2091, an accessory protein that enhances the immunogenicity of fHbp [31].

OMV (NZ98-254): Outer membrane vesicle (OMV) of Neisseria meningitidis strain NZ98-254. The main antigenic component of the OMV is PorA P1.4 [31].

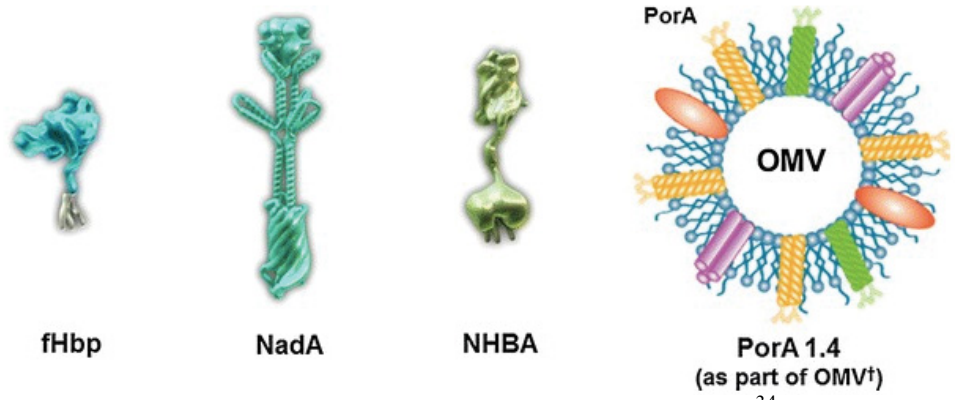


Fig. 3: Schematic representation of main Bexsero antigen components [34].

Why Bexsero: Bexsero was chosen for this study because of existing interest in understanding the human B cell response to its four main antigen components (fHbp, NadA, NHBA and PorA P1.4) [31]. Additionally, Bexsero antigens (fHbp, NadA and NHBA) were individually verified by Novartis to elicit robust antibody response post vaccination in rat or mouse models [31]. Serum Igs against each of these antigens were also detected in patients infected with *Neisseria meningitidis*. OMV (PorA P1.4) was also individually successfully used in a vaccine in New Zealand during a local infection by *Neisseria meningitidis* strain NZ98-254 [31]. These data confirmed the immunogenicity of each antigen individually and provided a basis to compare their immunogenicity when in a multi-complex.

***Neisseria meningitidis*:** It is a gram-negative bacterium, which colonizes the human nasopharynx. It persists asymptomatically in 10-40% of the human population and in a small but significant number of infections, the bacterium traverses the epithelium and reaches the bloodstream, causing septicemia [35,36]. Meningococci in the infected blood are able to cross the blood-brain barrier to infect the meninges, causing meningitis [37–39]. All currently known disease-causing meningococci posses a complex capsular polysaccharide wall [31]. There are 13 main serogroups defined by the chemical composition of their capsular polysaccharide, six

(serogroup A, B, C, Y, W135 and X) of which account for 95% of total cases of invasive meningococcal disease [31]. Serogroups A, X and W135 have mainly been found to cause epidemics in Africa. Serogroup B is primarily found in industrialized countries and serogroup C has been identified in epidemics worldwide [31]. Serogroup Y has been found only in North America [35]. Vaccines against serogroups A, C, Y, W135 and X were developed by using purified capsular polysaccharide as antigen [31]. However the chemical composition of serogroup B capsular polysaccharide is a polysialic acid similar to neural cell adhesion molecules, thus making it poorly immunogenic and presenting a possible cause of autoimmunity [40,41]. Novartis identified surface expressed protein antigens of *Neisseria meningitidis* serogroup B through reverse vaccinology to design Bexsero as a viable vaccine against serogroup B outbreaks.

Chapter 2: Results

Bexsero Vaccination and Vaccination Scheme

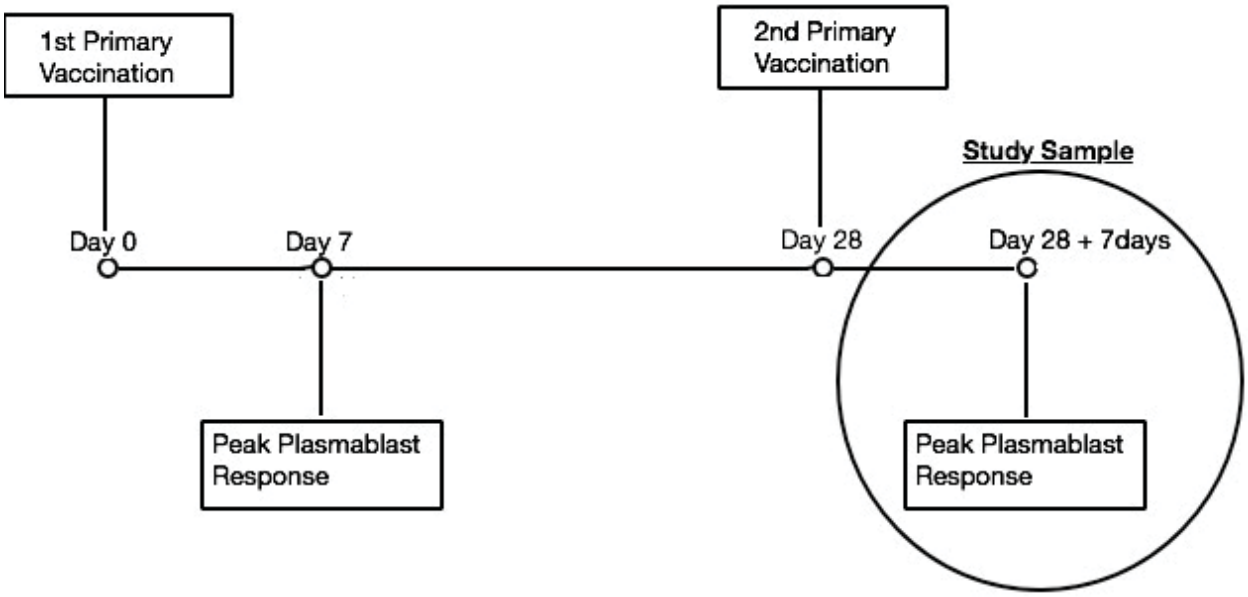


Fig.4: Vaccination scheme. Outline of Bexsero vaccination plan for the three vaccinees (Vax2, Vax3 and Vax4). **Day 0:** First primary vaccination time point **Day 7:** Peak plasmablast response time point after first primary vaccination **Day 28:** Second primary vaccination time point **Day (28 + 7):** Peak plasmablast response time after second primary vaccination. **Study sample:** Blood samples obtained for study.

Three healthy individuals (Adults) working in a laboratory setting with regular contact to multiple strains of *Neisseria meningitidis* were vaccinated with Bexsero, per required regulation, for protection against *Neisseria meningitidis* B infection. All three vaccinees volunteered to provide blood samples to address the questions elaborated in this project. The vaccination scheme followed the Novartis recommended vaccination plan of two Bexsero doses for adults, administered at least one-month apart [42]. The first and second primary vaccinations were offered to the vaccinees at a one-month interval. The outlined questions in this project necessitated assessing blood samples from the vaccinees at four different time points (pre-first primary vaccination (Day 0), 7 days post first primary vaccination, pre-second primary vaccination (Day 28) and 7 days post second primary vaccination to achieve a comprehensive study. Peripheral blood mononuclear cells (PBMCs) from blood samples obtained 7 days post vaccination are enriched in plasmablast [43], which enabled adequate assessment of the Igs generated against Bexsero.

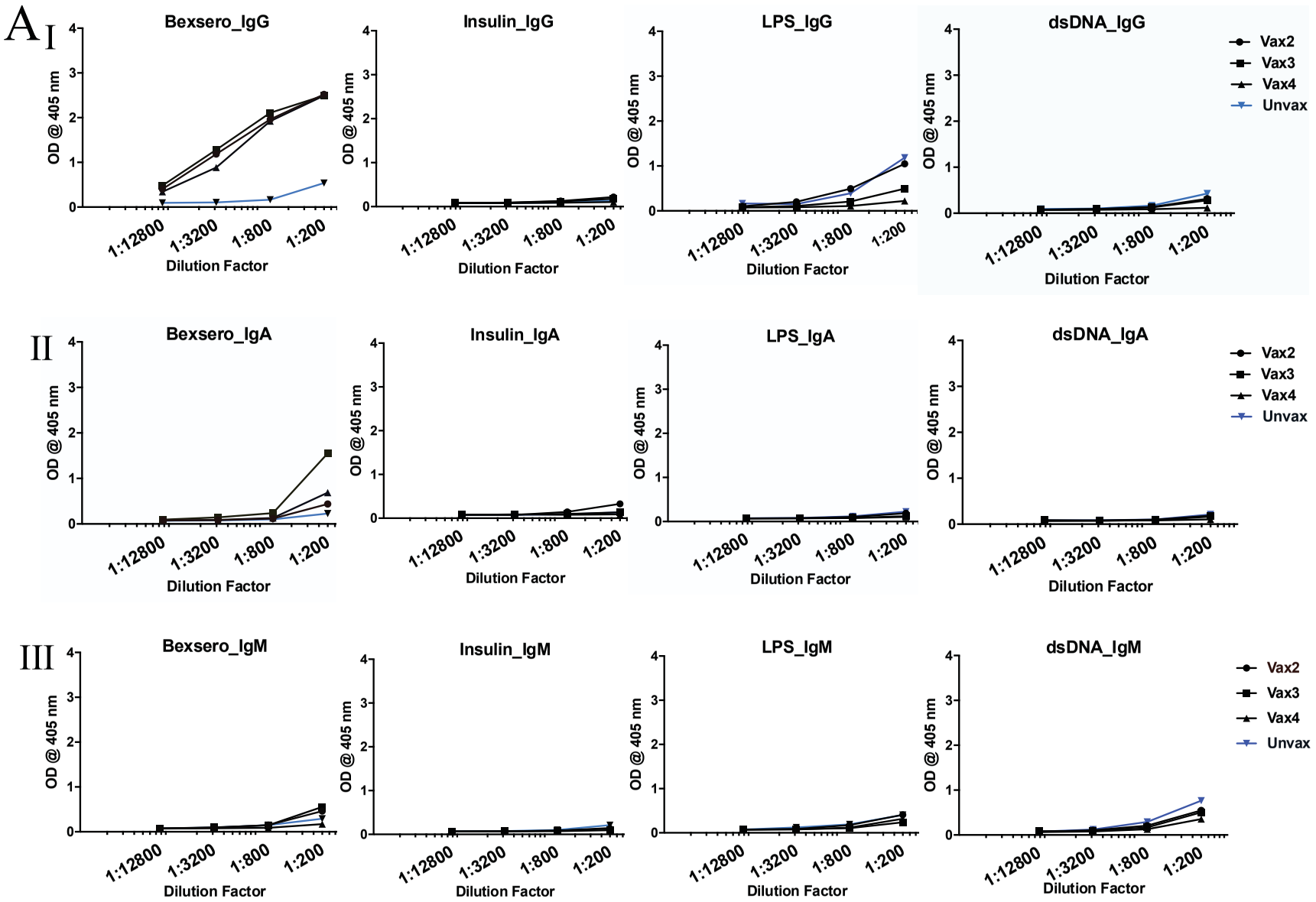
An outline of the vaccination scheme is depicted in figure 4. Pre-vaccination blood samples from Day 0 and Day 28 offer baseline cell populations to qualitatively and quantitatively analyze the plasmablast response; seven days post first and second primary vaccinations respectively. Organizational constraints disenabled obtaining all but blood samples from 7 days post second primary vaccination. Blood samples from seven days post second primary vaccination was therefore established as the study sample.

Serum ELISA and Western Blot Analysis Against Bexsero and fHbp

Sera from all three vaccinees (Vax2, Vax3 and Vax4) and an unvaccinated control sample (Unvax) were assessed for serum antibodies, to qualitatively ascertain success of the vaccination program, prior to working on the PBMC samples. To account for specificity of the interaction between serum anti-Bexsero antibodies and Bexsero antigens, the sera were also tested against three other randomly selected structurally diverse antigens (insulin, lipopolysaccharides (LPS) and double stranded DNA). Insulin, double stranded DNA and lipopolysaccharides were chosen primarily because they provide antigenic structural diversity to confirm polyreactivity properties of tested serum antibodies [44–46].

A predominant IgG anti-Bexsero antibody response in all three vaccinees (Vax2, Vax3 and Vax4) was observed in the ELISA data. In fig. 5A(I) sera from all vaccinees showed strong IgG anti-Bexsero antibody response compared to the unreactive Unvax serum. Sera from all vaccinees and Unvax were weakly reactive to LPS but unreactive to insulin and dsDNA. Anti-LPS antibodies detected, albeit very weakly in all three vaccinees may be a product of the natural propensity for exposure to LPS, common in most bacterial infections. The serum Ig reactivity to LPS may also be aspecific to both Bexsero vaccinees and Unvax sera. In fig. 5A(II) sera from all vaccinees but Unvax showed weak IgA anti-Bexsero antibody response against Bexsero but unreactive against insulin, LPS and dsDNA. Vax3 appeared to mount a stronger IgA anti-Bexsero response than Vax2 and Vax4. In fig. 5A(III) sera from Vax2 and Vax3 showed very weak IgM anti-Bexsero antibody response against Bexsero, LPS and dsNDA but unreactive against insulin whereas Vax4 and Unvax showed no IgM anti-Bexsero antibody reactivity to any of the four antigens. Serum antibodies against Bexsero antigens generally barely interact with insulin, LPS and double stranded DNA, affirming the specificity of the interaction between anti-Bexsero serum antibodies and Bexsero antigens. In fig. 5B, the anti-Bexsero serum antibodies were qualitatively assessed for antibodies against the unique Bexsero components (anti-NHBA-GNA1030 antibodies, anti-fHbp-GNA2091 antibodies, anti-NadA antibodies and anti-PorA P1.4 antibodies) in western blot. Fig. 5B(I) is an image of Coomasie stained (12%) gel after run of whole Bexsero vaccine (desorbed from $Al(OH)_3$ adjuvant) indicating the four main antigen components of Bexsero. The integrity of the desorbed antigens was verified by staining PVDF membrane blotted with whole Bexsero vaccine with mAB502 (mouse monoclonal antibody against fHbp: positive control) and mGO53 (in-house negative control antibody [44,47]) {fig. 5B(II)}. It appears desorption of Bexsero antigens from the $Al(OH)_3$ adjuvant had minimal effect on integrity of the vaccine antigens. A predominant IgG anti-fHbp-GNA2091 antibody response in all three vaccinees

(Vax2, Vax3 and Vax4) was observed (fig. 5BII). In fig. 5b(III) IgG anti-NHBA-GNA1030, anti-PorA P1.7-2,4 (very weak band) and anti-NadA (very weak band) antibody response were also observed in Vax3 but not Vax2 or Vax4 although the anti-PorA P1.7-2,4, and anti-NadA antibody response appeared very weak in sera of Vax3. In fig. 5C, anti-fHbp-GNA2091 antibodies in sera were further assessed to establish the target antigen (fHbp or GNA2091) to which serum anti-fHbp-GNA2091 bind. The ELISA data confirmed a strong specific IgG anti-fHbp serum antibody response in all three vaccinees {fig 5C(I)}, affirming that antibodies generated against fHbp-GNA2091 target fHbp and/or GNA2091. In fig. 5C(II), weak IgA anti-fHbp serum antibody response observed in all three vaccinees is shown whereas in fig. 5C(III), the lack of IgM anti-fHbp antibodies in sera of all vaccinees is shown. fHbp is the most immunogenic of the two-antigen conjugate whereas GNA2091 is a carrier protein introduced to enhance the immunogenicity of fHbp, hence the interest in anti-fHbp antibodies.


A
I
Bexsero_IgG
Insulin_IgG
LPS_IgG
dsDNA_IgG
II
Bexsero_IgA
Insulin_IgA
LPS_IgA
dsDNA_IgA
III
Bexsero_IgM
Insulin_IgM
LPS_IgM
dsDNA_IgM
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Dilution Factor
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1:3200
1:800
1:200
Vax2
Vax3
Vax4
Unvax

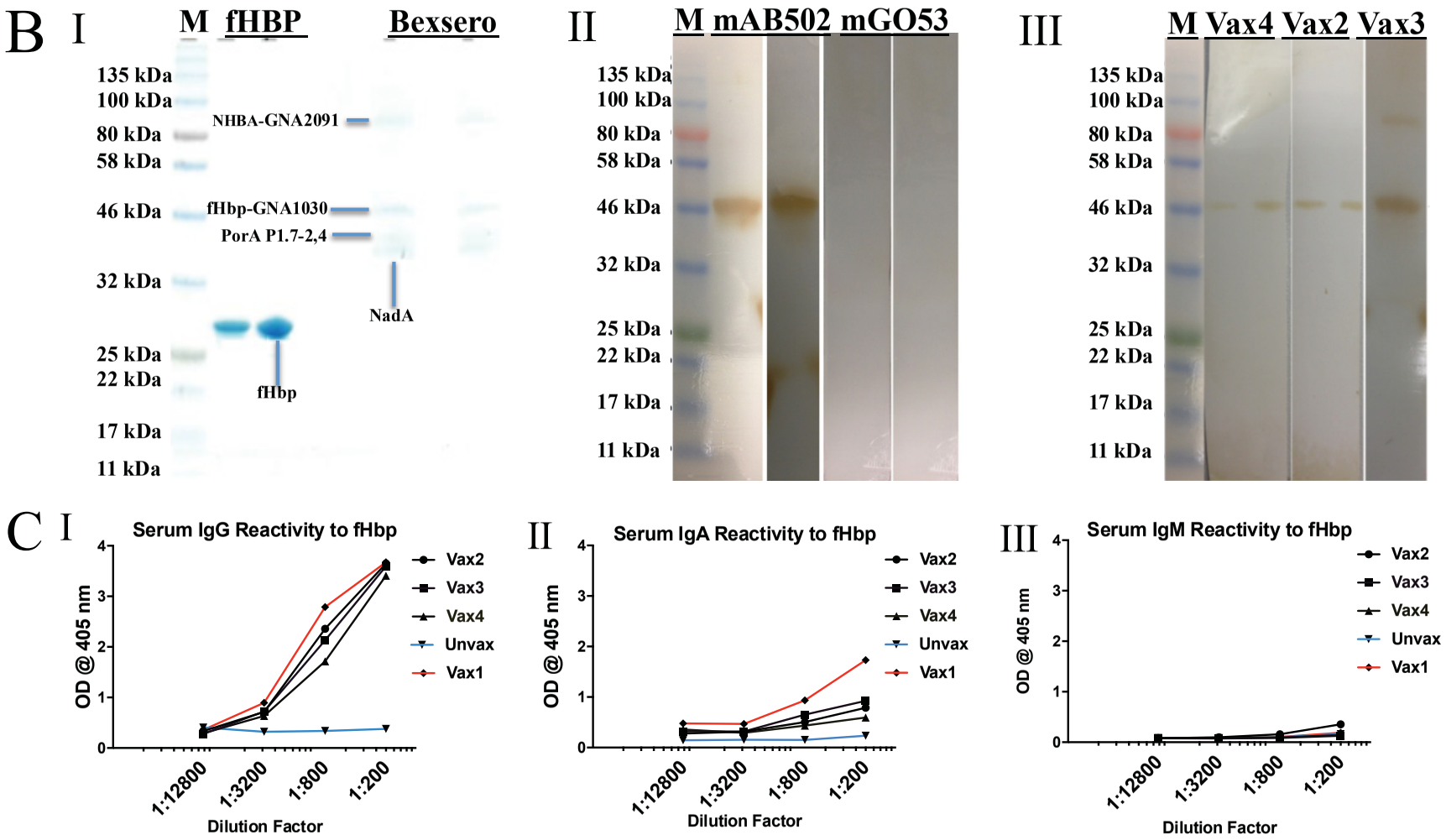


Fig. 5: Anti-Bexsero Serum Ig Analysis. A. Serum ELISA Analysis against Bexsero (I) Anti-Bexsero Serum IgG response to Bexsero, Insulin, LPS and dsDNA. **(II)** Anti-Bexsero Serum IgA response to Bexsero, Insulin, LPS and dsDNA. **(III)** Anti-Bexsero Serum IgM response to Bexsero, Insulin, LPS and dsDNA. Sera were diluted in a 1:4 dilution series at 1:200, 1:800, 1:3200 and 1:12800. **B: Serum Western Blot analysis against Bexsero**. **(I):** Gel image of fHbp (2 lanes) and whole Bexsero vaccine (desorb from $Al(OH)_3$ adjuvant (2 lanes) run on 12% gel and stained in Coomasie brilliant blue stain. **(II):** Image of PVDF membrane blotted with whole Bexsero antigens run on 12% gel and stained with mAB502 (2 lanes) and mG503 (2 lanes) **(II):** Image of PVDF membrane blotted with whole Bexsero antigens run on 12% gel and stained with sera from Vax2, Vax3 and Vax4 at 1:200 dilution. fHbp-GNA2091 = 47 kDA, NHBA-2091 = ~81 kDa, PorA P1.7-2, 4 = 42 kDa and NadA = 40 kDa. **5C: Serum ELISA analysis against fHbp.** (I) Serum IgG reactivity to fHbp. (II) Serum IgA reactivity to fHbp (III) Serum IgM reactivity to fHbp. **NB**: In fig. **5B(II)** and fig. **5B(III)**, photographic images of western blot PVDF membranes are shown instead of chemiluminescence images (not shown here) even though both images show exactly the same data.

FACS Analysis and Sorting Strategy of Plasmablast of Vaccinees

With evidence of specific serum antibodies against Bexsero antigens, plasmablast was sorted from PBMCs of all three vaccinees to assess the human B cell response to Bexsero antigens at the single cell level. Figure 6 depicts the gating strategy for sorting plasmablast ($CD19^{+}CD27^{+}CD38^{+}IgG^{-}$ cells) from Vax2, Vax3 and Vax4 PBMCs stained with combination of B cell markers CD19, CD27 and CD38 as well as IgG to selectively exclude memory B cells and other B cell intermediates/compartments that also express CD27 and/or CD38 from the plasmablast population [48,49]. The reason for the difference in plasmablast frequencies between Vax2 (fig.6D: 0.46 %), Vax3 (fig 6E: 3.76 %) and Vax4 (fig. 6F: 0.43 %) is unclear but appears to mirror the difference in quality observed in the serum data {(fig. 5B(III)}. A plate of 384 $CD19^{+}CD27^{+}CD38^{+}IgG^{-}$ plasmablast cells were sorted from Vax3 PBMCs, ½ of a 384 well plate of 192 cells from Vax2 and ¼ of a 384 well plate of 96 cells from Vax4, comparatively reflecting the impact of the observed differences in the frequencies of the Vax2, Vax3 and Vax4 plasmablast on the sorting efficiency.

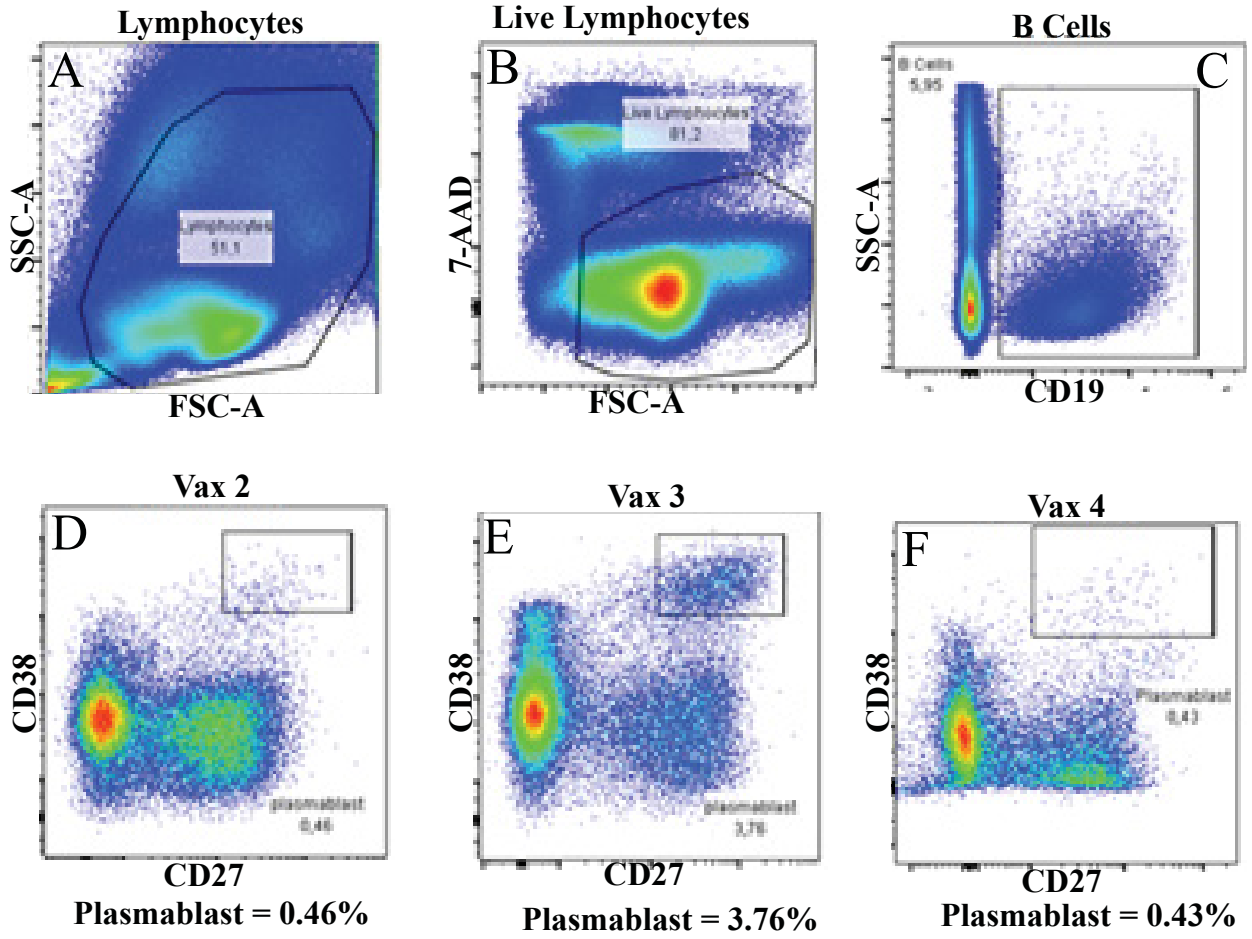


Fig. 6: Gating strategy for FACS analysis. (A)(B)(C): Representative plots of pre-plasmablast Vax3 gating analysis. **D**: Vax2 plasmablast gating, **E**: Vax3 plasmablast gating **F**: Vax4 plasmablast gating. Vax2:

Plasmablast frequency = 0.46% Vax3: Plasmablast frequency = 3.76% and Vax4: Plasmablast frequency = 0.43%

cDNA Synthesis and Single B Cell PCR

The single B cell antibody cloning is a technique that exploits fluorescence activated cell sorting (FACS) and polymerase chain reaction (PCR) technologies to copy and amplify Ig genes from the transcriptomes of sorted B cells, cloning into expression vectors for down stream antibody expression and purification [44]. Figure 7 (*see appendix*) is the collated PCR data of sorted plasmablast of Vax2, Vax3 and Vax4. The heavy chain amplification appears to be the least efficient, evidenced in Vax2, Vax3 and Vax4 plasmablast Ig genes amplification. ~ 15 – 22 % successfully amplified kappa and/or lambda chain PCR products in all vaccinees (Vax2, Vax3 and Vax4) were unused because of unamplified heavy chain matches. Combined matching heavy and light chain(s) amplification in the range of ~ 70 – 75 % with ~10% fall out frequency {all heavy and light chain(s) PCRs failed} in Vax2 and Vax4 appears to be very efficient. The Vax3 plate on the contrary drops in efficiency {combined matching heavy and light chain(s)} to ~ 42% with a relatively high fall out frequency of ~34.375%. The pronounced discrepancy between the two plates (Vax2: *IgH* = 75.5%, *Igk* = 62.0%, *Igλ* = 55.1%, *Igk/Igλ* = 91.1% and *IgH* + *Igk/Igλ* = 75.5% | Vax3: *IgH* = 43.25%, *Igk* = 40.1%, *Igλ* = 37.0%, *Igk/Igλ* = 65.6% and *IgH* + *Igk/Igλ* = 43.25% | Vax4: *IgH* = 73.0%, *Igk* = 64.7%, *Igλ* = 58.4%, *Igk/Igλ* = 88.7% and *IgH* + *Igk/Igλ* = 72.0%) is unclear given standardization in PCR conditions. Murugan et al, 2015 showed PCR efficiencies of *IgH* = 53%, *Igk* = 49%, *Igλ* = 30%, *Igk/Igλ* = 76% and *IgH* + *Igk/Igλ* = 41% for the PCR system used in this project [50]. Hence the observed data indicating enhanced PCR efficiency with an almost doubling effect in Vax2 and Vax4 was unexpected. The high efficiencies observed in Vax2 and Vax4 relative to Vax3 and the Murugan et al, 2015 data may be a statistical consequence of small sample sizes in Vax2 (192 cells) and Vax4 (96 cells) compared to Vax3 (384 cells). Alternatively, Murugan et al, 2015 established the PCR system on IgG^+ memory B cells, which may relatively express lesser amount of cytosolic Ig transcripts than activated antibody-secreting pre-plasma cell stage plasmablast, used in this project. This may account for the enhanced PCR efficiency in the Vax2, Vax3 and Vax4 Ig populations compared with the data of Murugan et al, 2015. Regardless, the PCR data appears to reflect the physiological kappa: lambda equilibrium of 2: 1. The high frequency of B cells with successfully copied heavy, kappa and lambda chains in Vax2 (23.4%) and Vax4 (31.3%) is unusual. This may be a consequence of false positive amplicons in one or more of the Ig chains of these Ig populations.

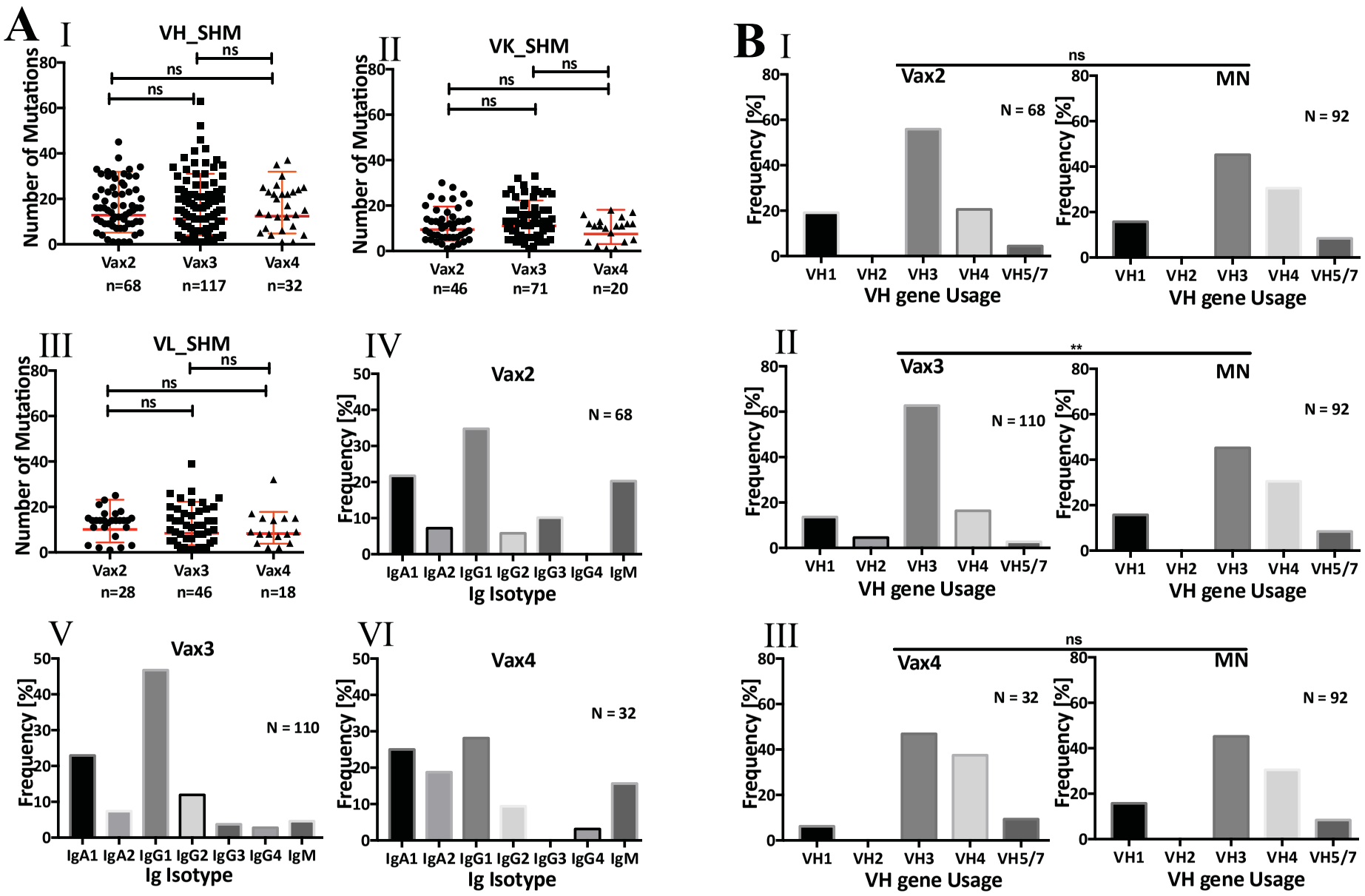
Sequence data

Ig gene sequences were analyzed using the Igblast (www.ncbi.nlm.nih.gov/igblast/) database to identify variable gene segments (heavy chain: V_H, kappa chain: V_K, and lambda chain: V_L), diversity gene segments (Heavy chain: D_H) and joining gene segments (heavy chain: J_H, kappa chain: J_K, and lambda chain: J_L). The somatic hypermutation (SHM) count of each Ig heavy and light chain and the complimentary determining region 3 (CDR3) amino acid sequence and length (amino acid count) of each Ig heavy chains were also collated. Using the ensembl (www.ensembl.org) database, the isotype of each heavy and light chain antibody sequence was annotated. Sequences from cells with successfully sequenced heavy and light chain genes were compiled for further analysis, table 15 (Vax3), table 16 (Vax2) and table 17 (Vax4) (*see tables in appendix*). 35.4% of all sorted Vax2 plasmablast (192 cells), 28.6% of all sorted Vax3 plasmablast (384 cells) and 46.9% of all sorted Vax4 plasmablast (96 cells) were successfully sequenced: heavy chains with their corresponding light chains. The frequency of antibodies expressing both kappa and lambda chains stood at 9.0% (Vax2), 7.3% (Vax3) and 13 % (Vax4) after sequencing, contrasting the unusually high frequencies observed in the PCR data (fig. 7: *see appendix*) of Vax2 (23.4 %) and Vax4 (31.3%). This observation supports the initial suspicion of false-positive amplicons as the drop out in the sequencing of these populations of antibodies was primarily observed in the lambda chains, hence pushing the kappa: lambda chain frequency equilibrium in Vax2 and Vax4 much closer to the physiological ratio of 2:1. Secondly, the observed frequencies of this population of antibodies in all three vaccinees after sequencing reflected the frequency (7.6 %) observed in the PCR data (fig. 7: *see appendix*) of Vax3.

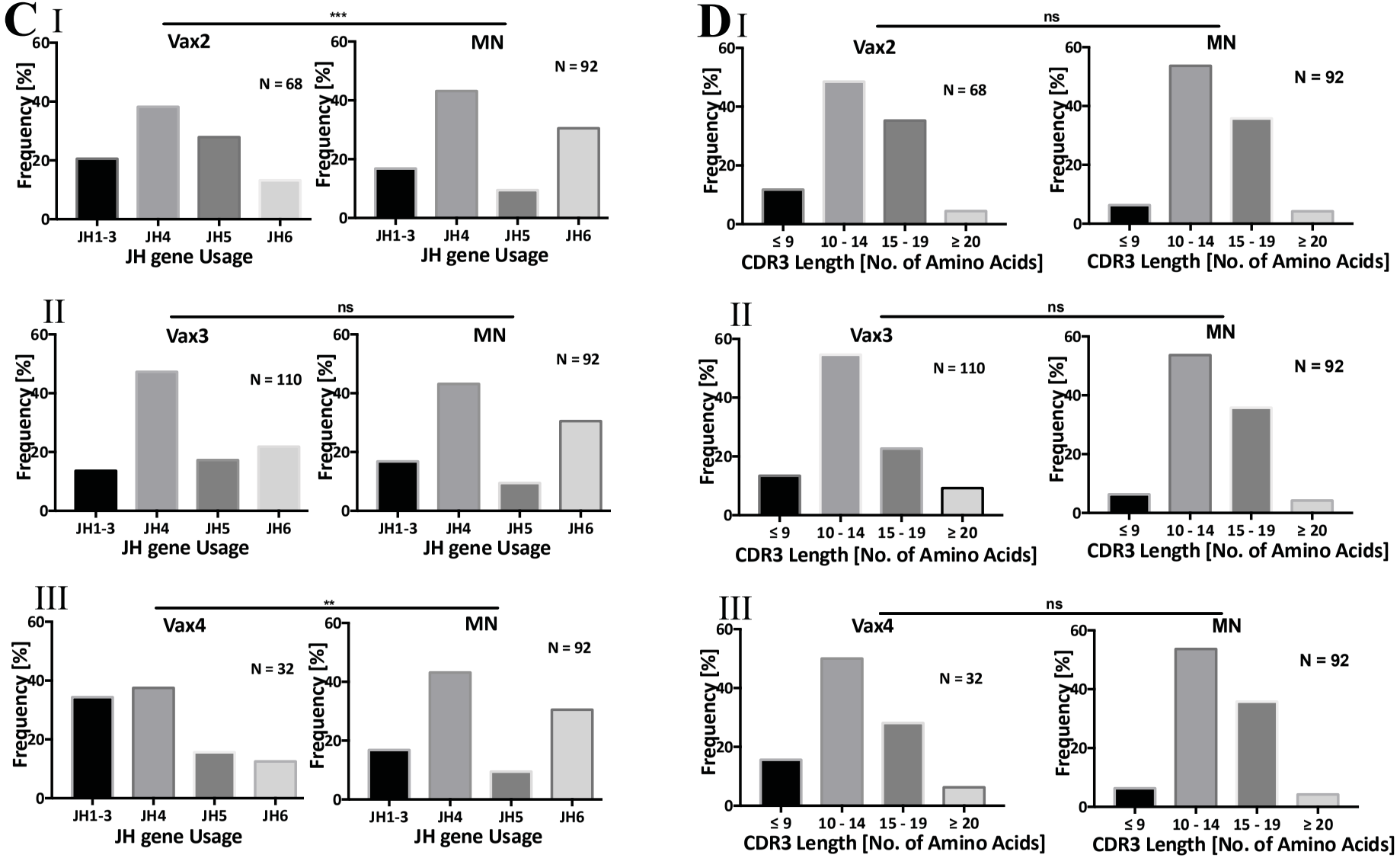
Analysis of Vax2, Vax3 and Vax4 Ig Gene Sequences

Ig genes were analyzed for any skews or unique selection patterns in the antibody repertoire recruited into the antibody response against Bexsero antigens. Ig heavy chain (V_H, J_H) and light chain (V_K/V_L, J_K/J_L) gene usage (frequency), SHM distribution, isotype distribution, and CDR3 length distribution were assessed. In fig. 8A(I), fig. 8A(II) and fig. 8A(III) respectively, the somatic hypermutation counts of Vax2, Vax3 and Vax4, were compared in VH, VK and VL genes. There was statistically, no significant difference in the accumulated mutations between Vax2, Vax3 and Vax4 in the heavy, kappa and lambda chain genes. This appears to suggest common behavior during affinity maturation in all three vaccinees B cell response to Bexsero. The isotype distribution of the Igs in all three vaccinees was also assessed {fig. 8A(IV), fig 8A(V) and fig. 8A(VI)}. IgG1 appeared to be predominantly

selected in all vaccinees at frequencies ranging between 30% and 45%, followed by IgA1 at ~20% in all vaccinees. In Vax2 [fig. 8A(IV)] and Vax4 [fig. 8A(VI)], IgM represents ~20% and 10% respectively of all Igs whereas IgA2 antibodies make up ~20% of Vax4 antibodies. In conclusion, the anti-Bexsero Ig response appeared mostly skewed towards IgG1 and IgA1. Vax2, Vax3 and Vax4 *IgVH* and *IgJH* genes were assessed in the Ig populations for selection patterns unique to the Bexsero vaccination (fig. 8B, fig. 8C and fig 8D). In comparison with mature naïve B cells (obtained from previous publications [44,51]), there appeared to be no distinct selection pattern in Vax2 *IgVH* {fig. 8B(I)} and Vax4 *IgVH* {fig. 8B(IIII)} genes but Vax3 *IgVH* {fig. 8B(II)} genes selected rarely used *IgVH2* and in general indicated a significant differential selection in *IgVH* genes. *IgJH* genes on the contrary indicated significant differential selection in Vax2 *IgJH* {fig. 8C(I)} and Vax4 *IgJH* {fig. 8C(III)} genes in comparison with the mature naïve Ig population whereas Vax3 *IgJH* {fig. 8C(II)} genes showed no significant selection pattern in comparison with mature naïve B cells. *IgVH* complimentary determining region 3 (CDR3) lengths were also analyzed in all three vaccinees for unique selection patterns in comparison with mature naïve B cells. No unique selection pattern was observed Vax2 {fig. 8D(I)}, Vax3 {fig. 8D(II)} and Vax4 {fig. 8D(III)} CDR3 length analysis. Vax2, Vax3 and Vax4 Ig gene sequences were also assessed for cluster antibodies {(fig. 8E | table 18 & table 19 (*see appendix*)}. Cluster antibodies originate from a common Ig lineage, sharing the same heavy and light chain genes, isotypes, same CDR3 length, same/similar CDR3 amino acid composition and same/variable numbers of somatic hypermutations. No cluster Igs were identified in Vax2, five and two clusters were identified in Vax3 and Vax4 respectively. Vax2 clusters were both made of two members each whereas Vax3 clusters ranged between two member clusters (cluster 1) to four member clusters (cluster 5). The frequency of clusters per vaccinee was calculated as part of the whole Ig population of the vaccinee.


A
I
VH_SHM
Number of Mutations
ns
Vax2 n=68
Vax3 n=117
Vax4 n=32
II
VK_SHM
Vax2 n=46
Vax3 n=71
Vax4 n=20
III
VL_SHM
Vax2 n=28
Vax3 n=46
Vax4 n=18
IV
Vax2
N = 68
Frequency [%]
IgA1 IgA2 IgG1 IgG2 IgG3 IgG4 IgM
Ig Isotype
V
Vax3
N = 110
VI
Vax4
N = 32
B
I
Vax2
N = 68
MN
N = 92
VH1 VH2 VH3 VH4 VH5/7
VH gene Usage
II
Vax3
N = 110
**
III
Vax4
N = 32

Continued


C
I
***
Vax2
N = 68
MN
N = 92
Frequency [%]
JH1-3
JH4
JH5
JH6
JH gene Usage
II
ns
Vax3
N = 110
III
**
Vax4
N = 32
D
I
ns
≤ 9
10 - 14
15 - 19
≥ 20
CDR3 Length [No. of Amino Acids]
II
III

Continued

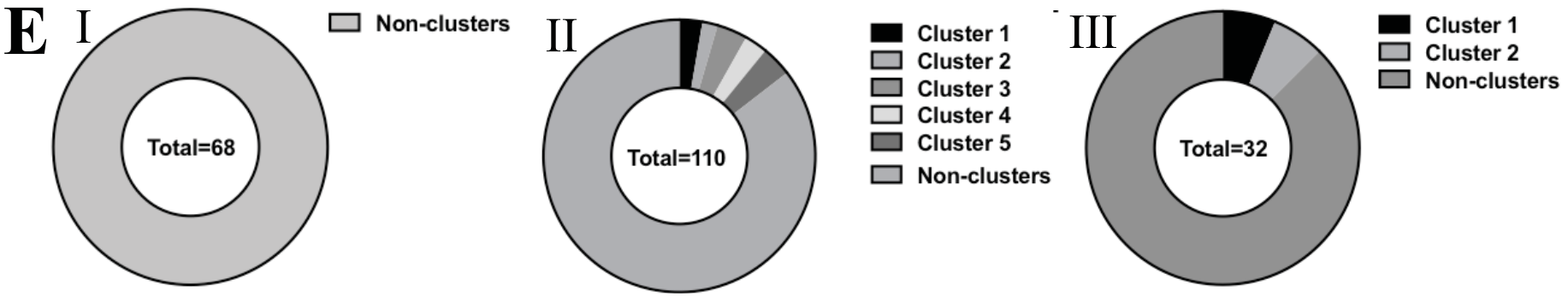
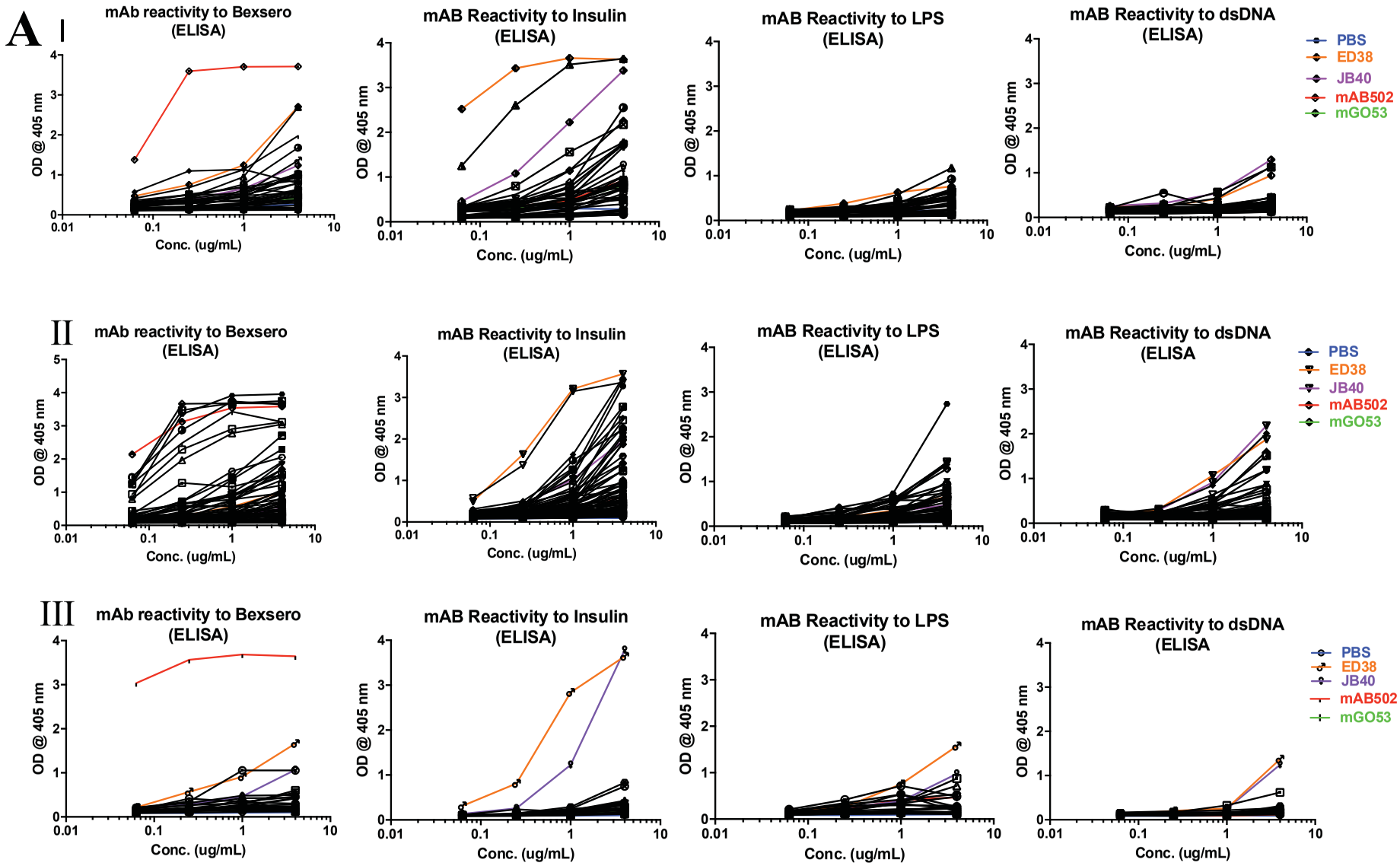


Fig. 8: Ig Gene Analysis A: Somatic hypermutation (nucleotides) in *IgVH, IgVK* & *IgVL* genes. **(I)** Plot comparing Vax2, Vax3 and Vax4 *IgVH* somatic hypermutations. **(II)** Plot comparing Vax2, Vax3 and Vax4 *IgVK* somatic hypermutations. **(III)**: Plot comparing Vax2, Vax3 and Vax4 *IgVL* somatic hypermutations. The number of Ig sequences analyzed for each Vaccinee is indicated. P-values were determined using student's t-test. Geometric means with SD and the absolute numbers of sequences analyzed are indicated. **B: Isotype distribution of Igs of Vax2, Vax3 and Vax4.** **(I)**: Bar graph of the distribution of frequencies [%] of Ig isotypes in Vax2. **(II)**: Bar graph of the distribution of frequencies [%] of Ig isotypes in Vax3. **(III)**: Bar graph of the distribution of frequencies [%] of Ig isotypes in Vax4. Frequencies were calculated as percentages of total Ig populations. **C: Ig VH gene selection: (I)**: Bar graphs comparing *IgVH* gene selection between Vax2 and mature naïve B cells. **(II)**: Bar graphs comparing IgVH gene selection between Vax3 and mature naïve B cells. **(III)**: Bar graphs comparing *IgVH* gene selection between Vax4 and mature naïve B cells. Frequencies were calculated as percentages of total Ig populations. P-values were determined using Fischer's exact test. **D: Ig JH gene selection: (I)**: Bar graphs comparing *IgJH* gene selection between Vax2 and mature naïve B cells **(II)**: Bar graphs comparing *IgJH* gene selection between Vax3 and mature naïve B cells. **(III)**: Bar graphs comparing *IgJH* gene selection between Vax4 and mature naïve B cells. P-values were determined using Fischer's exact test. **E: Ig CDR3 length analysis: (I)**: Bar graphs comparing *IgVH* CDR3 length analysis between Vax2 and mature naïve B cells. **(II)**: Bar graphs comparing *IgVH* CDR3 length analysis between Vax3 and mature naïve B cells **(III)**: Bar graphs comparing *IgVH* CDR3 length analysis between Vax4 and mature naïve B cells. Frequencies were calculated as percentages of total Ig populations. P-values were determined using Fischer's exact test. **F: Ig cluster analysis: (I)**: Frequencies of Vax2 cluster Igs calculated as part of a whole set of Igs. **(II)**: Frequency of Vax3 cluster Igs calculated as part of a whole set of Igs. **(III)**: Frequency of Vax4 cluster Igs calculated as part of a whole set of Igs.

Monoclonal Antibody Reactivity Properties (ELISA)

Vax2, Vax3 and Vax4 antibodies expressed in HEK293T cells were tested against Bexsero in ELISA. To account for specificity of the interaction between monoclonal antibodies and Bexsero antigens, the antibodies were also tested against three other randomly selected structurally diverse antigens (insulin, lipopolysaccharides (LPS) and double stranded DNA). Vax2 antibodies showed a distribution of moderately binding, weakly binding and unreactive anti-Bexsero antibodies, {fig. 9A (I)}. In the insulin plate, Vax2 comprised of moderately binding, weakly binding and unreactive antibodies {fig. 9A (I)}. In both LPS and dsDNA plates, Vax2 Igs comprised of weakly binding and unreactive antibodies {fig. 9A (I)}. Vax3 antibodies showed a distribution of highly binding, moderately binding, weakly binding and unreactive anti-Bexsero antibodies {fig. 9A (II)}. In the insulin plate, Vax3 Igs comprised of highly binding, moderately binding, weakly binding and unreactive antibodies {fig. 9A (II)}. In both LPS and dsDNA plates, Vax3 Igs comprised of moderately binding, weakly binding and unreactive antibodies {fig. 9A (II)}. Vax4 antibodies showed a distribution of poorly binding and unreactive antibodies against Bexsero, insulin, LPS and dsDNA {fig. 9A (III)}. Fig 9A (I), (II) and (III) were repeated in triplicates. The "area under curve" values for the reactivity of each antibody to Bexsero, insulin, LPS and dsDNA were also determined. The bar charts in fig 9B (I), (II), (III), (IV) and (V) indicate average area under curve (AUC) values (3X) of the ELISA data for Vax2 {fig. 9B(I)(II)}, Vax3 {fig. 9B(III)(IV)} and Vax4 {fig. 9B(V)}, antibodies. The reactivity of each antibody to each of the four antigens (Bexsero, insulin, LPS and dsDNA) was determined in relation to the reactivity properties of PBS (background reactivity), mGO53 (negative control monoclonal antibody unreactive to Bexsero, insulin, LPS and dsDNA), ED38 (highly polyreactive antibody), JB40 (moderately polyreactive antibody) and mAB502 (mouse monoclonal antibody against fHbp: positive control) [44–47]**.** Antibodies with AUC values below the blue line (mGO53 reactivity) were classified as unreactive antibodies. Antibodies with AUC values between the blue and red lines were classified as potentially weak binding or unreactive antibodies. All antibodies with AUC values above the red line were classified as reactive antibodies. Antibodies were established as polyreactive if they showed reactivity to at least two of the randomly selected antigens (insulin, LPS and dsDNA)[44–46]. Tables 20 (Vax2), 21 (Vax4) and 22 (Vax3) details the list of antibodies determined to be reactive to Bexsero in ELISA and their respective Ig gene properties.


A
I
mAb reactivity to Bexsero (ELISA)
mAB Reactivity to Insulin (ELISA)
mAB Reactivity to LPS (ELISA)
mAB Reactivity to dsDNA (ELISA)
OD @ 405 nm
Conc. (ug/mL)
PBS
ED38
JB40
mAB502
mGO53
II
mAb reactivity to Bexsero (ELISA)
mAB Reactivity to Insulin (ELISA)
mAB Reactivity to LPS (ELISA)
mAB Reactivity to dsDNA (ELISA)
OD @ 405 nm
Conc. (ug/mL)
PBS
ED38
JB40
mAB502
mGO53
III
mAb reactivity to Bexsero (ELISA)
mAB Reactivity to Insulin (ELISA)
mAB Reactivity to LPS (ELISA)
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OD @ 405 nm
Conc. (ug/mL)
PBS
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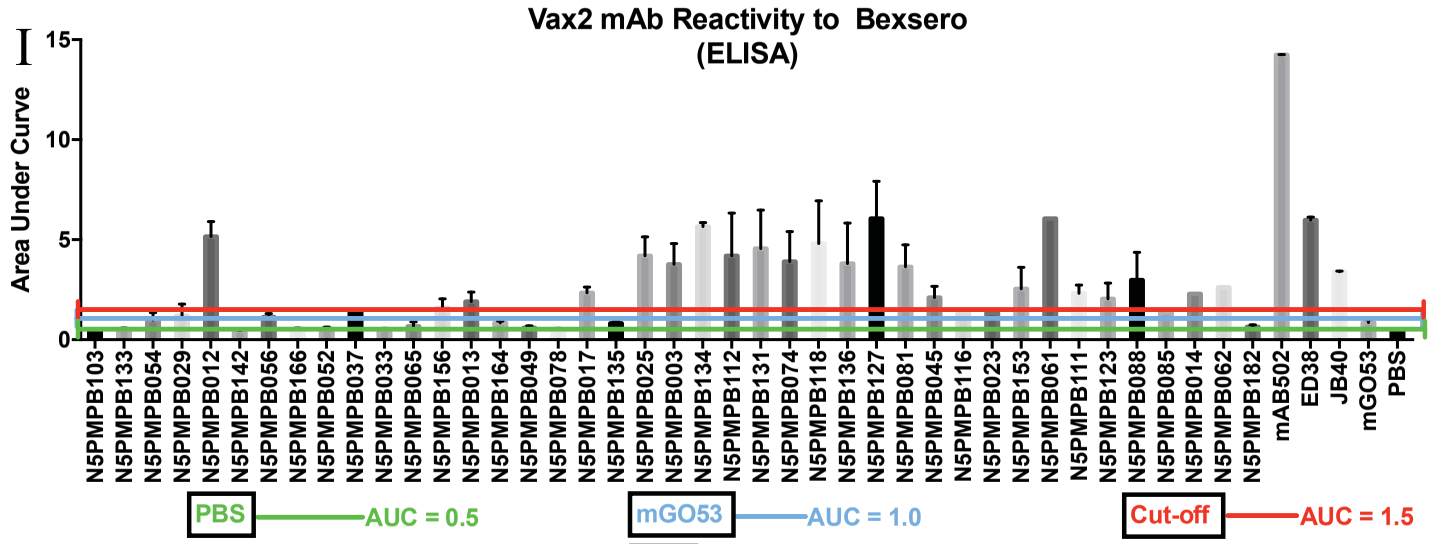
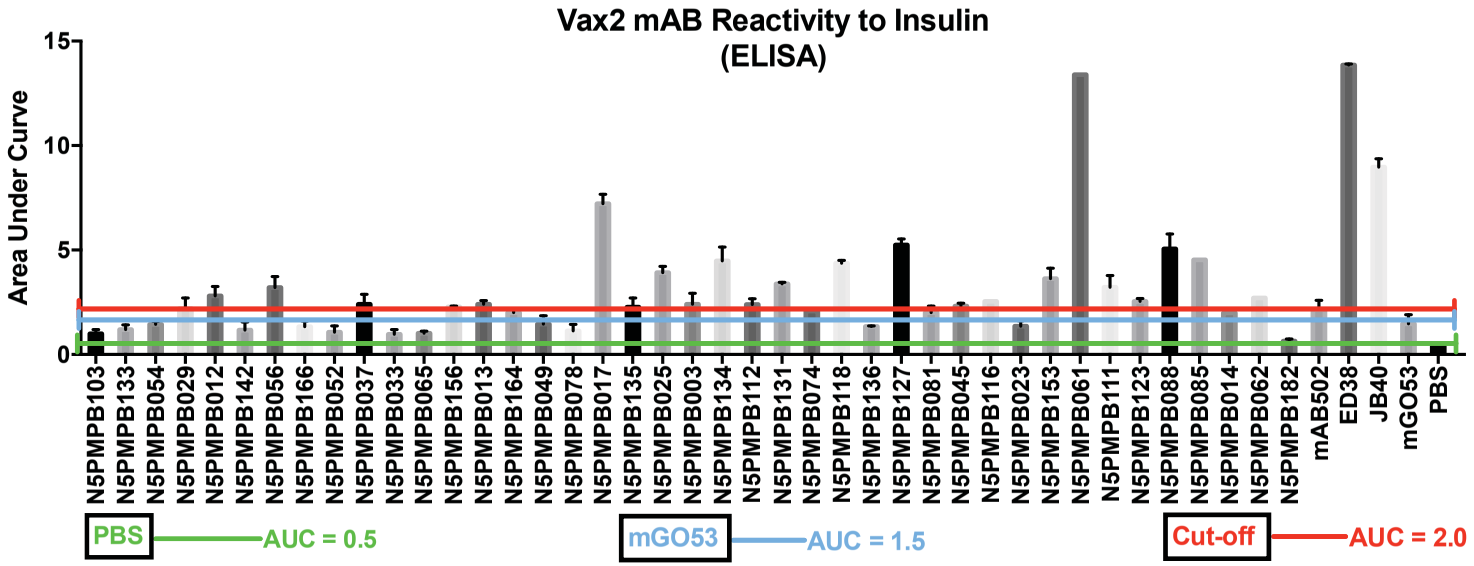
mAB502
mGO53

B I





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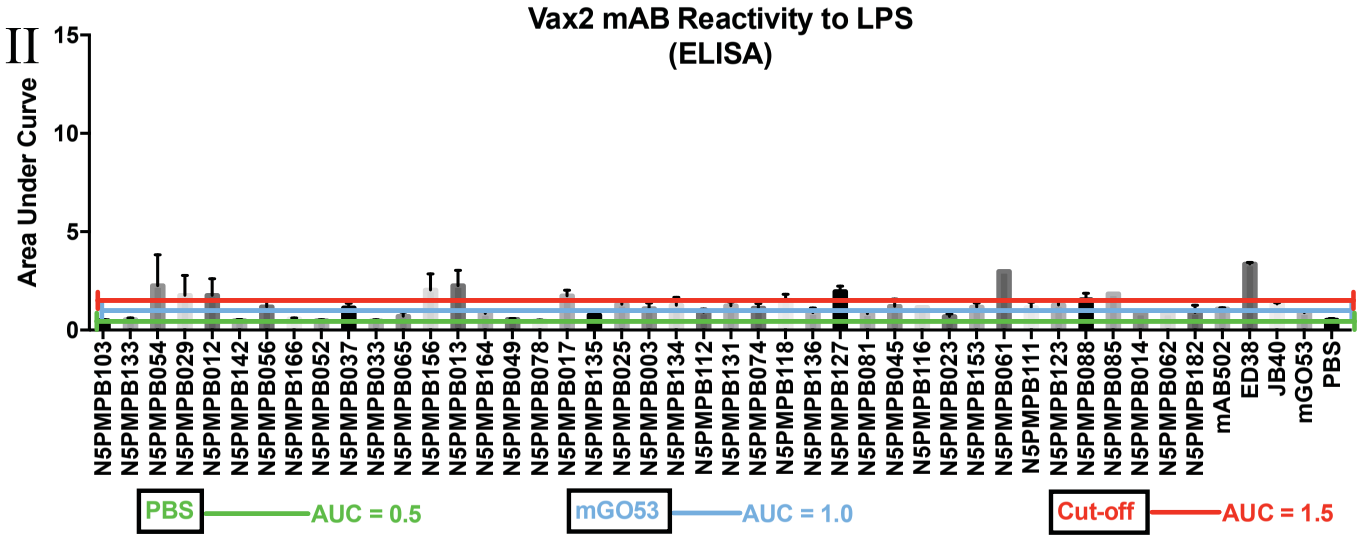
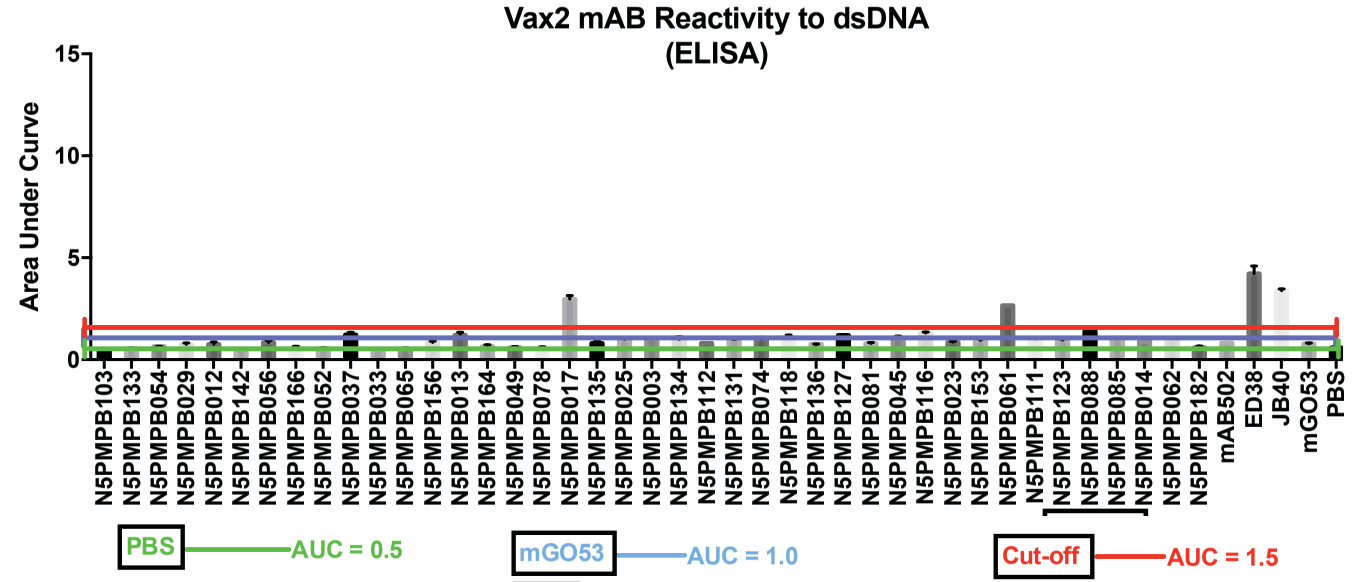
II





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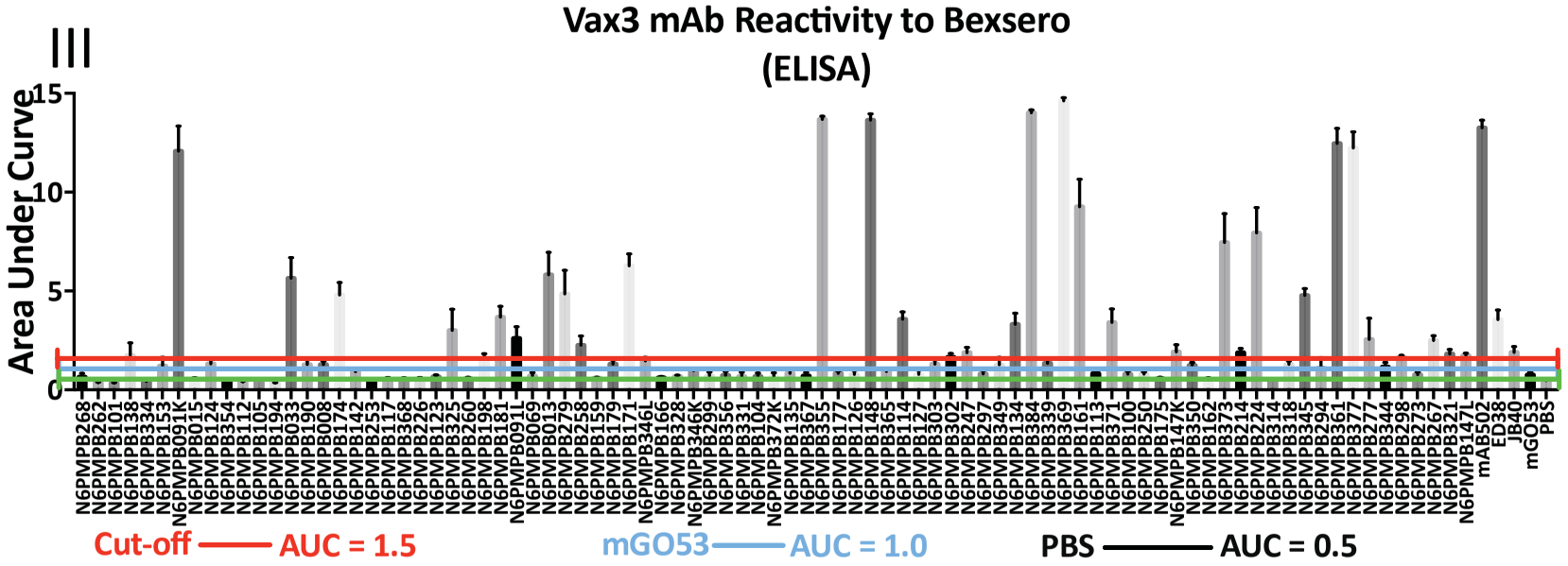

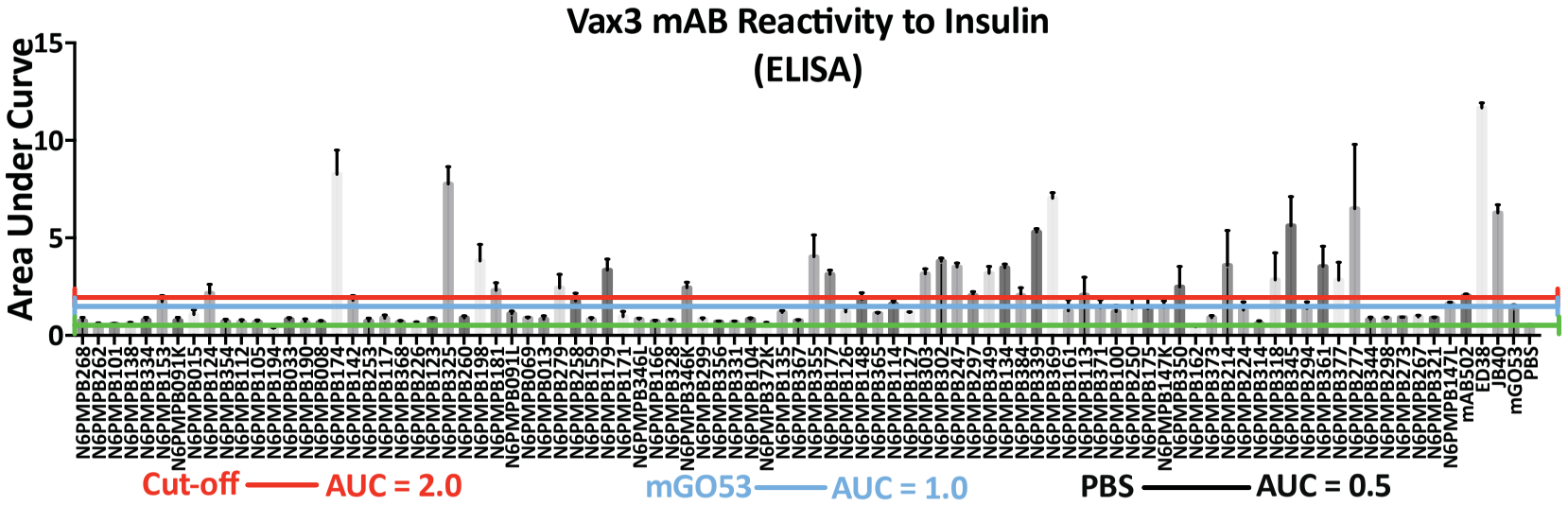

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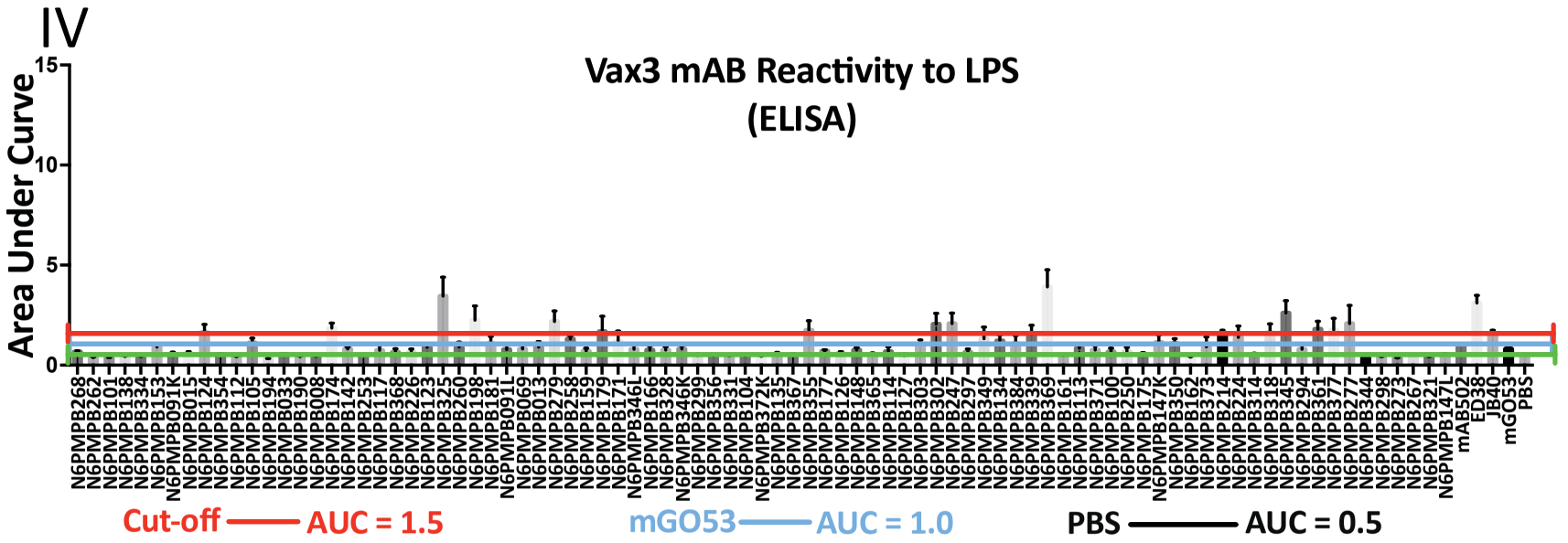

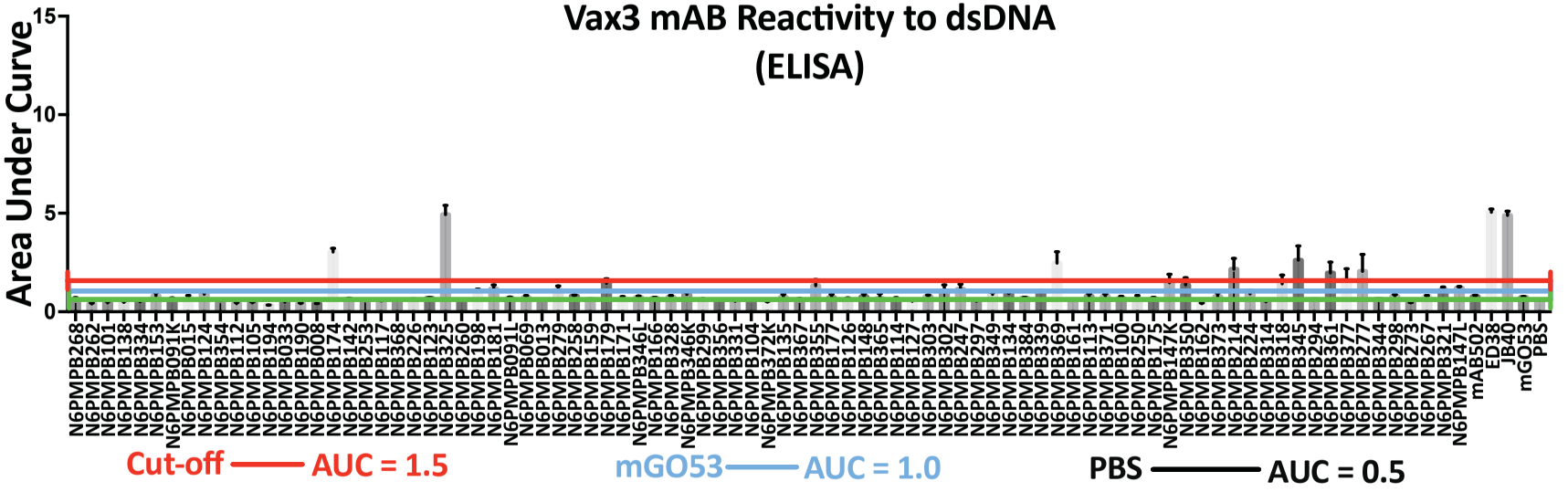

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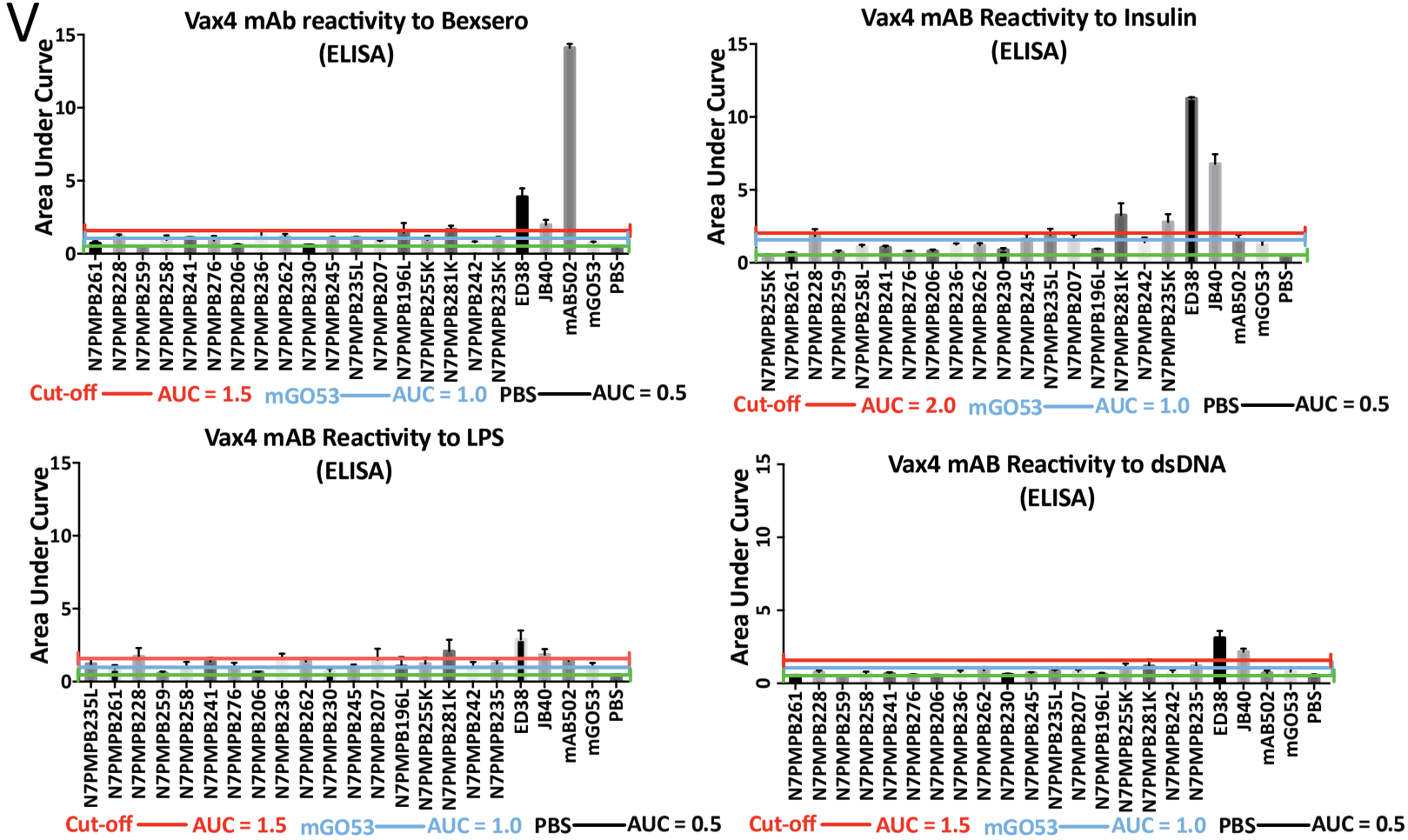
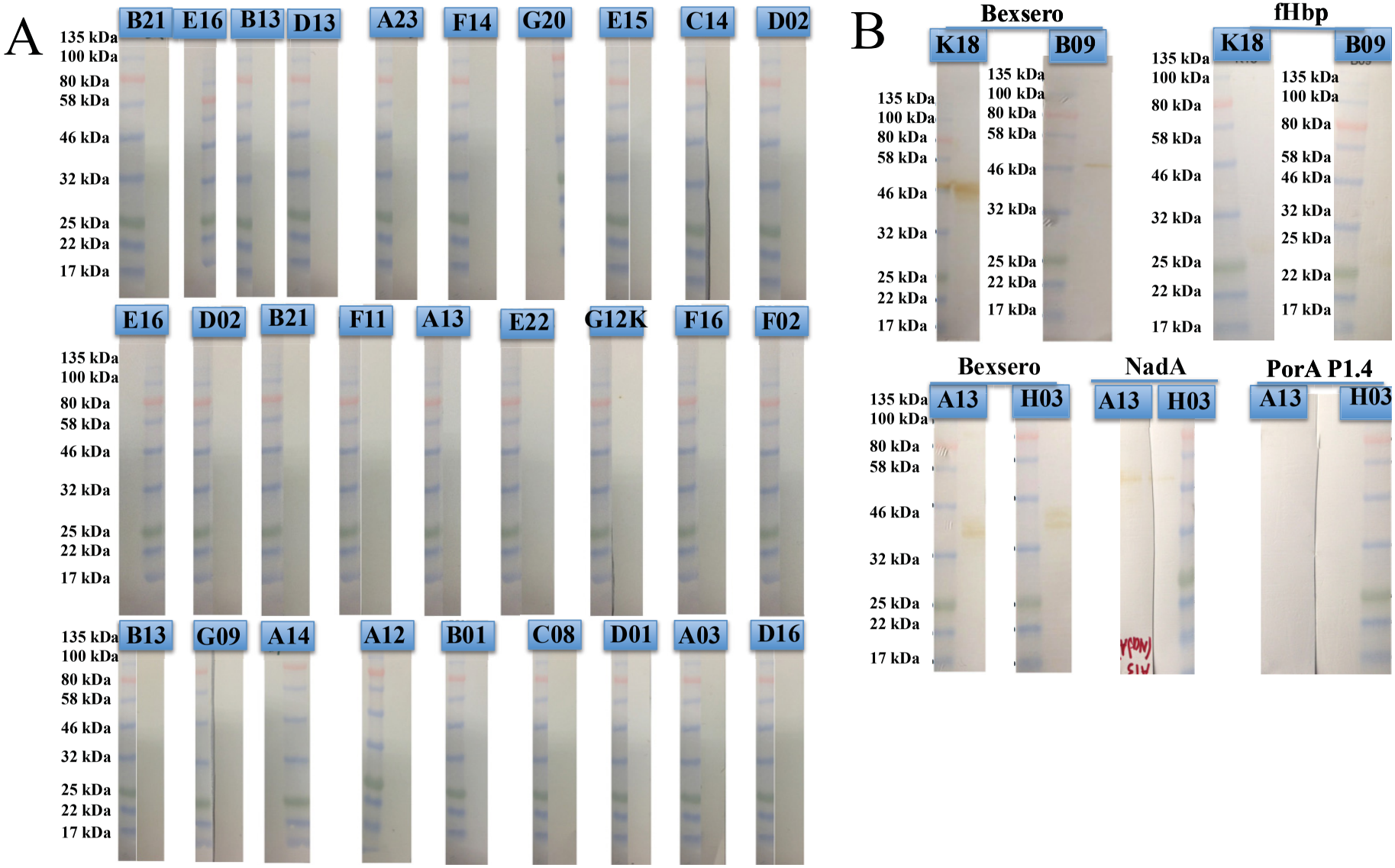


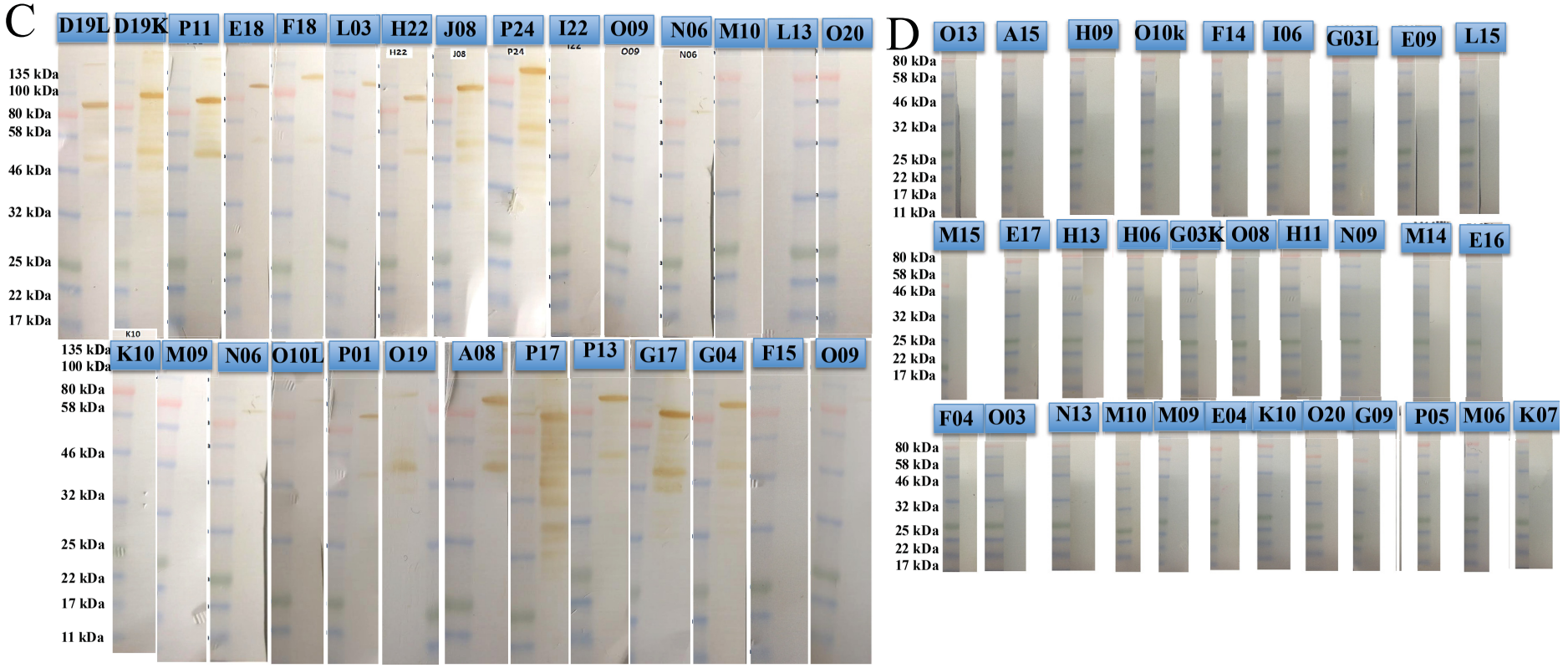
Fig. 9 A: Monoclonal antibody reactivity (ELISA). (I) Vax2 Igs reactivity to Bexsero, Insulin, LPS and dsDNA **(II)** Vax3 Igs reactivity to Bexsero, Insulin, LPS and dsDNA **(III)** Vax4 Igs reactivity to Bexsero, Insulin, LPS and dsDNA. **(I), (II)** and **(III)** were repeated in triplicates. Antibodies with reactivity values (OD) above the reactivity values of mGO53 and PBS were classified to be reactive to the respective antigen. Antibodies with reactivity values (OD) equal or below the reactivity values mGO53 and PBS were classified as unreactive to the respective antigen. **B: Monoclonal antibody reactivity (ELISA). (I) & (II):** Bar graphs of average AUC values of Vax2 Igs reactivity to Bexsero, insulin, LPS and dsDNA. **(III) & (IV):** Bar graphs of average AUC values of Vax3 Igs reactivity to Bexsero, insulin, LPS and dsDNA. **(V):** Bar graphs of average AUC values of Vax4 Igs reactivity to Bexsero, insulin, LPS and dsDNA. PBS reactivity (AUC =0.5) measures background reactivity, mGO53 reactivity indicates the reactivity of negative control monoclonal antibody unreactive to Bexsero (4CmenB). AUC values below the blue line (AUC=1.0) were classified as unreactive antibodies. Igs with AUC values between the blue and red lines were classified as potential weak binding or unreactive Igs. Igs with AUC values above the red line were classified as reactive Igs.

Monoclonal Antibody Reactivity to Bexsero (Western Blot Analysis)

All antibodies identified to be reactive to Bexsero in ELISA were analyzed on western blot (5ug of antibody: 1ug of antigen) to determine the specific antigen to which they bind. The antibody: antigen amounts used in western blot is ~ 10-fold higher than the amounts used in the ELISA experiments in Fig. 9a (0.4ug of antibody | 0.1ug of antigen), increasing the resolution range (10X) to significantly improve odds of identifying relatively weakly binding antibodies. All Vax2 (Fig. 10A) and Vax4 (Fig. 10E) antibodies failed to interact with Bexsero antigens in western blot. Two Vax3 antibodies bind to fHbp-GNA1030, two Vax3 antibodies bind to NadA and twenty-two Vax3 antibodies bind to NHBA-GNA2091, (fig. 10B and fig 10C | table 23). Thirty-one Igs were unreactive to any of the Bexsero component antigens (fig. 10D). Tables 24 (Vax2), 25 (Vax3) and 26 (Vax4) are the lists of all antibodies of each vaccinee and their respective reactivity (Bexsero, Insulin, LPS and dsDNA) properties (values measured at OD 405 nm) (*see appendix*). The data in tables 24, 25 and 26 are mean OD values calculated from three separate ELISA experiments with standard error of the mean (SEM). In relation to the sampling frequency, 6.8% (26/384), 6.8% (26/384) and 10.2% (39/384) antibodies were reactive, polyreactive and unreactive Vax3 antibodies respectively. Additionally, 0% (0/192), 13.5% (26/192) and 7.8% (15/192) of Vax2 antibodies were reactive, polyreactive and unreactive Vax3 antibodies respectively. Vax4 antibodies at frequencies 0% (0/96), 16.7% (16/96) and 2% (2/96) were reactive, polyreactive and unreactive respectively. Vax3 antibodies reactive to Bexsero in ELISA but not western blot were mostly weakly binding polyreactive antibodies (fig. 10F).


A
B21
E16
B13
D13
A23
F14
G20
E15
C14
D02
135 kDa
100 kDa
80 kDa
58 kDa
46 kDa
32 kDa
25 kDa
22 kDa
17 kDa
E16
D02
B21
F11
A13
E22
G12K
F16
F02
B13
G09
A14
A12
B01
C08
D01
A03
D16
B
Bexsero
K18
B09
fHbp
Bexsero
A13
H03
NadA
PorA P1.4

Continued


C
D19L
D19K
P11
E18
F18
L03
H22
J08
P24
I22
O09
N06
M10
L13
O20
135 kDa
100 kDa
80 kDa
58 kDa
46 kDa
32 kDa
25 kDa
22 kDa
17 kDa
K10
M09
N06
O10L
P01
O19
A08
P17
P13
G17
G04
F15
O09
11 kDa
D
O13
A15
H09
O10k
F14
I06
G03L
E09
L15
M15
E17
H13
H06
G03K
O08
H11
N09
M14
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K10
O20
G09
P05
M06
K07

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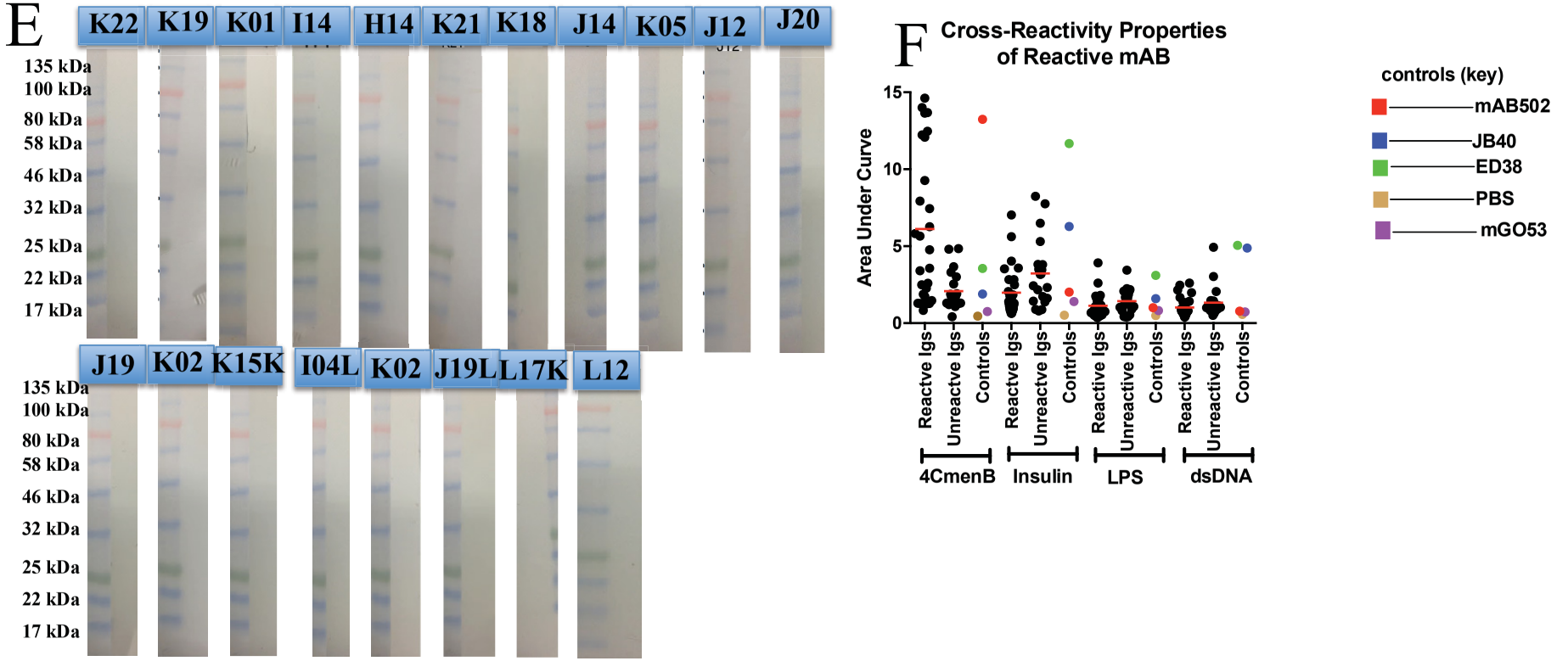
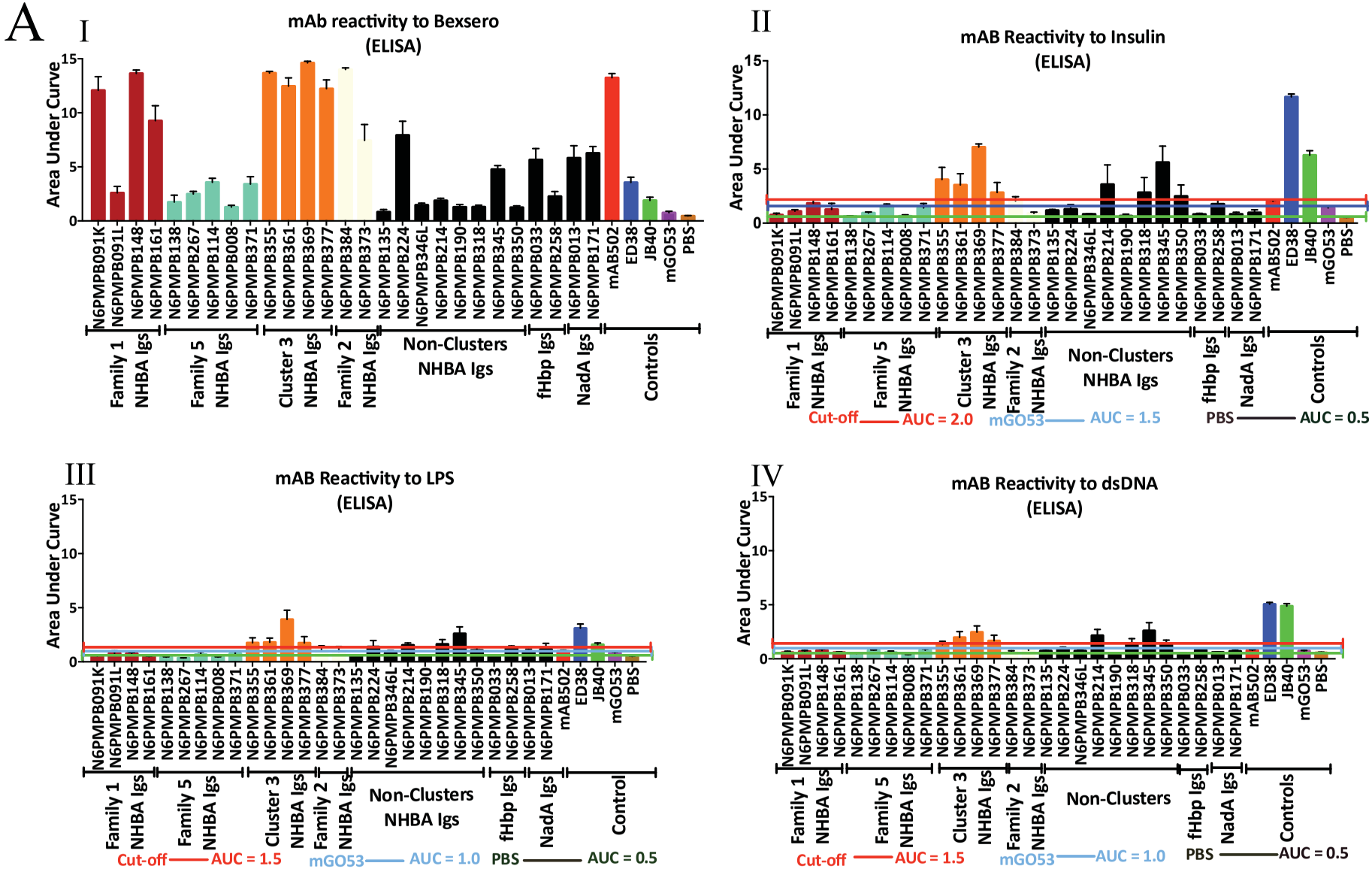


Fig. 10 Monoclonal antibody reactivity (Western blot) A: Vax2 Igs reactivity to Bexsero. **B:** Vax3 Bexsero-reactive Igs specific to fHbp component of fHbp-GNA1030 (K18: N6PMPB258 and B09: N6PMPB033) and Vax3 Bexsero-reactive Igs specific to NadA (A13: N6PMPB013 and H03: N6PMPB171. **C:** Vax3 Igs reactive to NHBA. The antibodies bind to both NHBA-GNA2091 at ~81 kDa and second band at ~ 51 kDa (NHBA). **D:** Vax3 Igs unreactive to any Bexsero antigen. **E:** Vax4 Igs reactivity to Bexsero. Photographic images of western blot PVDF membranes are shown here instead of chemiluminescence images (not shown here) even though both images are exactly the same data. See tables 30, 31 and 32 (in *appendix*) for legend pairing Ig IDs with Ig names. **F: Analysis of Bexsero-reactive Igs.** Comparison of the cross-reactive properties of Vax3 Igs reactive to Bexsero in both ELISA and western blot with Vax3 Igs reactive to Bexsero in ELISA but unreactive in western blot.

Ig Reactivity Properties and Gene Features of Bexsero-Specific-Reactive Igs

Igs identified to be reactive to Bexsero in both ELISA and western blot screens were re-assessed for their reactivity properties, Ig gene family usage patterns, somatic hypermutation, isotype distribution and *IgH* CDR3 features. Anti-NHBA-GNA2091 antibodies collectively disproportionately constituted 84.6% (22/26) of all reactive antibodies compared to 7.69% (2/26) anti-fHbp-GNA1030 antibodies and 7.69% (2/26) anti-NadA antibodies (fig. 11A). Cluster Igs (cluster 1, cluster 2, cluster 3 and cluster 5) also disproportionately constituted 63.6% (14/22) of all anti-NHBA-GNA2091 Igs in comparison to 36.4% (8/22) non-cluster anti-NHBA-GNA2091 Igs (fig. 11A). The Ig response to the second primary vaccination appeared to be predominantly targeted to NHBA-GNA2091. Cluster 3 Igs; N6PMPB355, N6PMPB361 and N6PMPB377 reacted weakly to insulin whereas N6PMPB369 reacts moderately to insulin and LPS (fig. 11A). The Ig gene features of this cluster only differed slightly by the SHM load and single amino acid differences in the CDR3 sequences of both heavy and light chains. This may account for the differences in their polyreactivity properties. N6PMPB091, which appears related to Igs in cluster 1 expresses both kappa (N6PMPB091_K) and lambda (N6PMPB091_L) variants with a strongly binding kappa variant and weakly binding lambda variant. The reactivity property of N6PMPB091 therefore appears to be highly dependent on the constitution of its light chain Ig gene properties. Non-cluster Ig, N6PMPB345 reacted moderately to insulin and weakly to LPS and dsDNA (fig. 11A). Non-cluster Ig, N6PMPB214 also reacted weakly to LPS and dsDNA (fig. 11A). All other Bexsero-reactive Igs appeared to be specific to Bexsero and did not react with any of the other select antigens (Insulin, LPS and dsDNA). In figure 11B (I), SHM of the Vax3 Bexsero-specific reactive Ig population was compared with the unreactive Vax3 Ig population. The reactive Vax3 Igs comprised of moderately mutated and lowly mutated Ig populations (V_H, V_K and V_L). Comparatively, the unreactive Vax3 Ig population comprised of highly mutated, moderately mutated and poorly mutated Ig populations (V_H, V_K and V_L). In fig 11B (II), the isotype distribution of Vax3 Bexsero-specific reactive Ig population was compared with the unreactive Vax3 Ig population. Bexsero-reactive Vax3 Igs did not select IgA2, IgG4 and appeared to have down-regulated IgM selection when compared with the unreactive Vax3 Ig population. In general, there is no distinct significant differential selection pattern in the isotype distribution of the Bexsero-specific reactive Vax3 Igs in the overall anti-Bexsero response. Analysis of *IgVH* genes in fig. 11B(III) showed selection of rarely used *IgVH2*, and a general significant differential selection of *IgVH genes* in comparison with the mature naïve B cell population. The significant *IgVH* differential selection was more

pronounced when compared with the unreactive Vax3 Ig population, which displays a mostly homogenous usage of *IgVH3* {fig. 11B(IV)}. The Bexsero-reactive Vax3 Ig populations *IgJH* also showed significant differential selection of genes when compared with the mature naïve population with a pronounced skew towards rarely used JH1, JH2 and JH3 genes {fig. 11B(V)}. On the contrary, there is no unique significant selection pattern when compared with the unreactive Vax3 Ig population {fig. 11B(VI)}. The CDR3 length usage distribution also displayed a significant differential selection of varied CDR3 lengths when compared with the mature naïve Ig population {fig. 11B(VII)} but not the unreactive Ig population {fig. 11B(VIII)}. Regarding antibody:antigen interaction, anti-NHBA Igs comprised of high binding Igs and weak binding Igs. Both populations exhibited a mix of both lowly mutated and highly mutated Igs. The weak binding Igs predominantly used *IgVH3* gene whereas *IgVH1*, rarely used *IgVH2* and *IgVH4* Ig gene usage was observed in the high binding Igs. Rarely used *IgVH7* was used in one of the anti-fHbp Igs (N6PMPB033) that is moderately mutated and of the IgA1 isotype. The other anti-fHbp Ig (N6PMPB258) and the two anti-NadA Igs (N6PMPB013 and N6PMPB171) are lowly mutated and use *IgVH3* gene. N6PMPB013 is an IgA whereas Igs N6PMPB258 and N6PMPB171 are IgG1 Igs. Both populations also exhibit diversity in isotype distribution.


A
I
mAb reactivity to Bexsero
(ELISA)
Area Under Curve
N6PMPB091K
N6PMPB091L
N6PMPB148
N6PMPB161
N6PMPB138
N6PMPB267
N6PMPB114
N6PMPB008
N6PMPB371
N6PMPB355
N6PMPB361
N6PMPB369
N6PMPB377
N6PMPB384
N6PMPB373
N6PMPB135
N6PMPB224
N6PMPB346L
N6PMPB214
N6PMPB190
N6PMPB318
N6PMPB345
N6PMPB350
N6PMPB033
N6PMPB258
N6PMPB013
N6PMPB171
mAB502
ED38
JB40
mGO53
PBS
Family 1
NHBA Igs
Family 5
NHBA Igs
Cluster 3
NHBA Igs
Family 2
NHBA Igs
Non-Clusters
NHBA Igs
fHbp Igs
NadA Igs
Controls
II
mAB Reactivity to Insulin
(ELISA)
Cut-off
AUC = 2.0
mGO53
AUC = 1.5
PBS
AUC = 0.5
III
mAB Reactivity to LPS
(ELISA)
Control
IV
mAB Reactivity to dsDNA
(ELISA)

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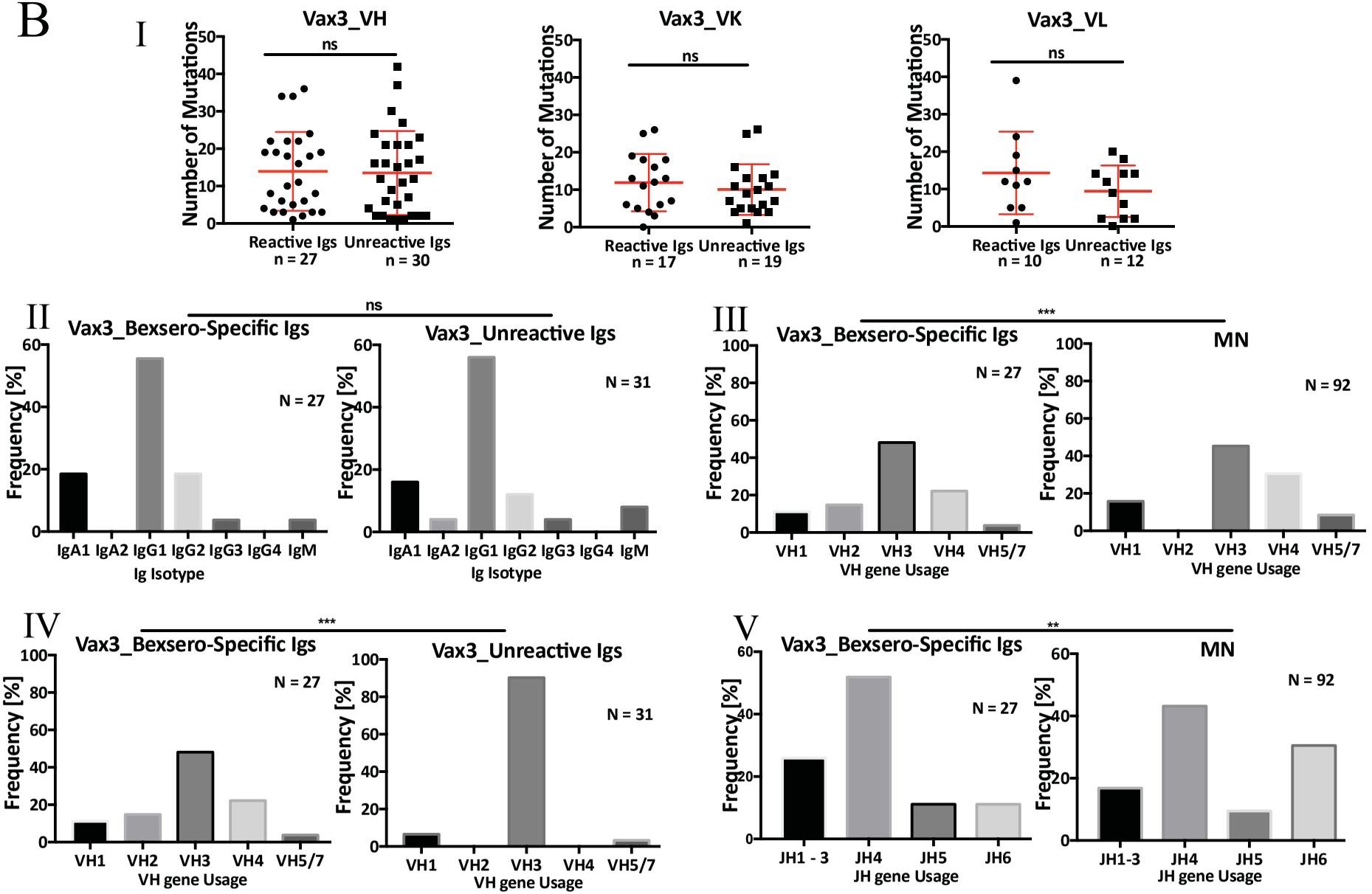
B
I
Vax3_VH
ns
Number of Mutations
Reactive Igs n = 27
Unreactive Igs n = 30
Vax3_VK
ns
Number of Mutations
Reactive Igs n = 17
Unreactive Igs n = 19
Vax3_VL
ns
Number of Mutations
Reactive Igs n = 10
Unreactive Igs n = 12
II
ns
Vax3_Bexsero-Specific Igs
N = 27
Frequency [%]
IgA1 IgA2 IgG1 IgG2 IgG3 IgG4 IgM
Ig Isotype
Vax3_Unreactive Igs
N = 31
Frequency [%]
IgA1 IgA2 IgG1 IgG2 IgG3 IgG4 IgM
Ig Isotype
III
***
Vax3_Bexsero-Specific Igs
N = 27
Frequency [%]
VH1 VH2 VH3 VH4 VH5/7
VH gene Usage
MN
N = 92
Frequency [%]
VH1 VH2 VH3 VH4 VH5/7
VH gene Usage
IV
***
Vax3_Bexsero-Specific Igs
N = 27
Frequency [%]
VH1 VH2 VH3 VH4 VH5/7
VH gene Usage
Vax3_Unreactive Igs
N = 31
Frequency [%]
VH1 VH2 VH3 VH4 VH5/7
VH gene Usage
V
**
Vax3_Bexsero-Specific Igs
N = 27
Frequency [%]
JH1 - 3 JH4 JH5 JH6
JH gene Usage
MN
N = 92
Frequency [%]
JH1-3 JH4 JH5 JH6
JH gene Usage

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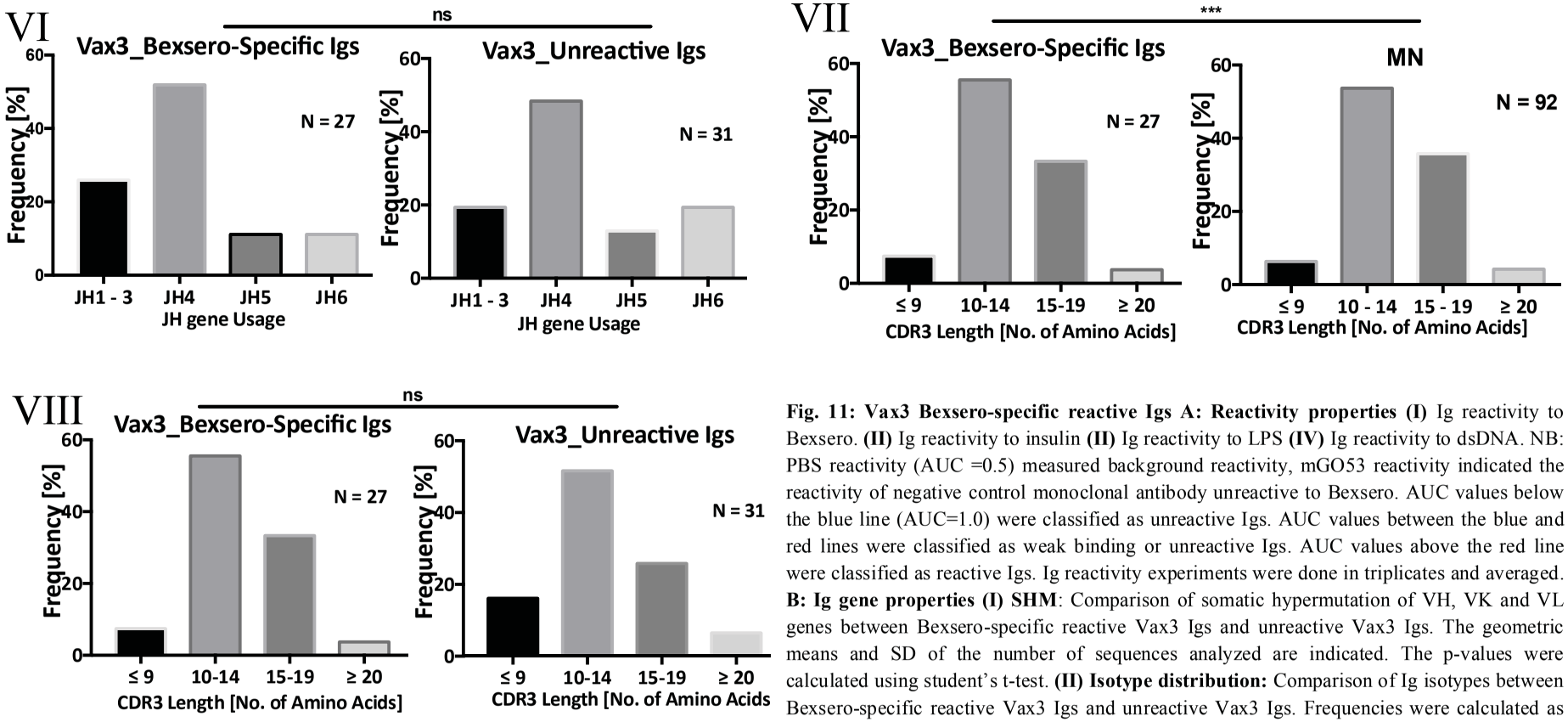


Fig. 11: Vax3 Bexsero-specific reactive Igs A: Reactivity properties (I) Ig reactivity to Bexsero. **(II)** Ig reactivity to insulin **(II)** Ig reactivity to LPS **(IV)** Ig reactivity to dsDNA. NB: PBS reactivity (AUC =0.5) measured background reactivity, mGO53 reactivity indicated the reactivity of negative control monoclonal antibody unreactive to Bexsero. AUC values below the blue line (AUC=1.0) were classified as unreactive Igs. AUC values between the blue and red lines were classified as weak binding or unreactive Igs. AUC values above the red line were classified as reactive Igs. Ig reactivity experiments were done in triplicates and averaged. **B: Ig gene properties (I) SHM**: Comparison of somatic hypermutation of VH, VK and VL genes between Bexsero-specific reactive Vax3 Igs and unreactive Vax3 Igs. The geometric means and SD of the number of sequences analyzed are indicated. The p-values were calculated using student's t-test. **(II) Isotype distribution:** Comparison of Ig isotypes between Bexsero-specific reactive Vax3 Igs and unreactive Vax3 Igs. Frequencies were calculated as percentages of total Ig population. P-values were calculated using Fishers exact test.

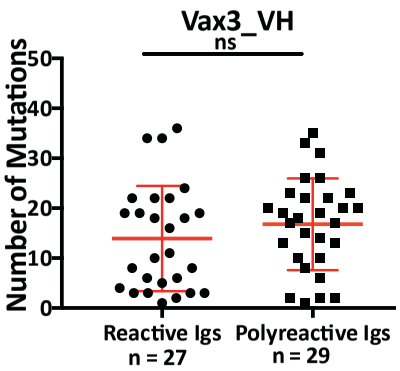
(III) VH Repertoire: Comparison of VH repertoire between Bexsero-specific reactive Vax3 Igs and mature naive Igs. Frequencies were calculated as percentage of total Ig population. P-values were calculated using Fishers exact test. **(IV) VH Repertoire:** Comparison of VH repertoire between Bexsero-specific reactive Vax3 Igs and unreactive Vax3 Igs. Frequencies were calculated as percentage of total Ig population. P-values were calculated using Fishers exact test. **(V) JH Repertoire:** Comparison of JH repertoire between Bexsero-specific reactive Vax3 Igs and mature naive Igs. Frequencies were calculated as percentage of total Ig population. P-values were calculated using Fishers exact test. **(VI) JH Repertoire:** Comparison of JH repertoire between Bexsero-specific reactive Vax3 Igs and unreactive Vax3 Igs. Frequencies were calculated as percentage of total Ig population. P-values were calculated using Fishers exact test. **(VII) Ig CDR3 length:** Comparison of CDR3 length usage between Bexsero-specific reactive Vax3 Igs and mature naiveVax3 Igs. Frequencies were calculated as percentage of total Ig population. P-values were calculated using Fishers exact test. **(VIII) Ig CDR3 length:** Comparison of CDR3 length usage between Bexsero-specific reactive Vax3 Igs and unreactive Vax3 Igs. Frequencies were calculated as percentage of total Ig population. P-values were calculated using Fishers exact test.t.

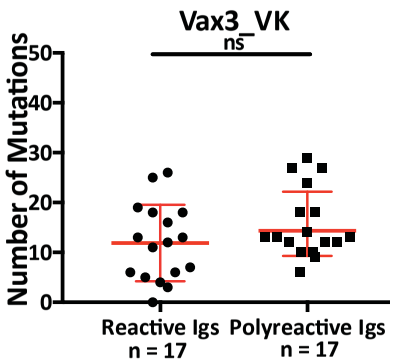
Ig Gene Analysis and Reactivity Properties of Polyreactive Antibodies

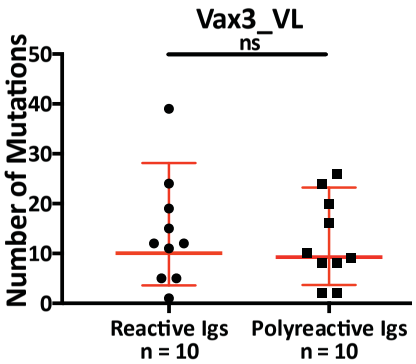
The polyreactive B cell populations of Vax2, Vax3 and Vax4 were analyzed for gene usage patterns, SHM, isotype distribution, CDR3 length distribution and reactivity properties in comparison with the gene features of the identified Bexsero-antigen specific reactive B cell, mature naïve B cell and unreactive B cell populations. It is important to note that Vax4 are mostly weakly binding antibodies to Bexsero as well as to insulin, LPS and dsDNA. They were therefore classified as polyreactive, not only based on their reactivity properties but also their Ig gene features in comparison with polyreactive Igs of Vax2 and Vax3. The lists of Vax2, Vax3 and Vax4 polyreactive Igs are shown in tables 27, 28 and 29 respectively. In fig. 12A (I) Vax3 polyreactive Igs SHM were compared with the unreactive Vax3 Igs VH, VK and VL genes whereas in fig 12A(II), Vax3 polyreactive Igs SHM were compared with the Bexsero-specific reactive Igs. In fig. 12A(III), Vax2 polyreactive Igs SHM in VH, VK and VL genes were compared with unreactive Vax3 Igs. No significant differential selection was observed in SHM distribution in both Vax2 and Vax3 polyreactive Igs. However, the polyreactive Igs appear to have averagely accumulated more mutations in *IgVH* and *IgVK* genes of all vaccinees compared to the unreactive Igs. In fig. 12A(IV), fig. 12A(V) and fig. 12A(VI), *IgVH* gene selection of Vax2, Vax3 and Vax4 respectively, were compared with mature naïve B cells. A significant differential *IgVH* gene selection was observed in Vax2 {fig. 12A(IV)} but not Vax3 {fig. 12A(V)} and Vax4 {fig. 12A(VI)}. A similar comparison was done for *IgJH* gene selection and Ig CDR3 length usage. Significant differential selection in *IgJH* genes was observed in Vax2 {fig. 12A(VII)}, Vax3 {fig. 12A(VIII)} and Vax4 {fig. 12A(IX)} in comparison with mature naïve B cells. On the contrary, significant differential selection in Ig CDR3 length usage was observed in Vax3 {fig. 12A(XI)} and Vax4 {fig. 12A(XII)} but not Vax2 {fig. 12A(X)}. In fig. 12B, the *IgVH* gene usage, *IgJH* gene usage and Ig CDR3 length usage of polyreactive Vax2 and Vax3 Igs were compared with their corresponding unreactive Igs. Vax4 was excluded due to the small size of the Vax4 unreactive Ig population. A significant differential selection of *IgVH* genes was observed in both Vax2 {fig. 12B(I)} and Vax3 {fig. 12B(II)} whereas significant differential selection of IgJH genes was observed in Vax3 {fig. 12B(IV)} but not Vax2 {fig. 12B(III)}. In fig. 12B(V) and fig 12B(VI), significant differential selection of CDR3 length was observed respectively in Vax2 and Vax3 polyreactive Igs. The isotype distribution of Vax2 and Vax3 polyreactive Igs was compared with unreactive and Bexsero-specific reactive Igs (Vax3 only). No significant differential selection of Ig isotypes was observed in Vax3 polyreactive Igs when compared with Vax3 Bexsero-specific reactive Igs {fig. 12C(III)} whereas in

comparison with their respective unreactive Igs, significant differential selection of Ig isotypes was observed in Vax2 Igs {fig. 12C(I)} but not Vax3 Igs {fig. 12C(II)}.

A I







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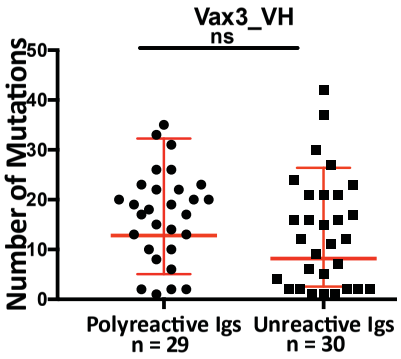

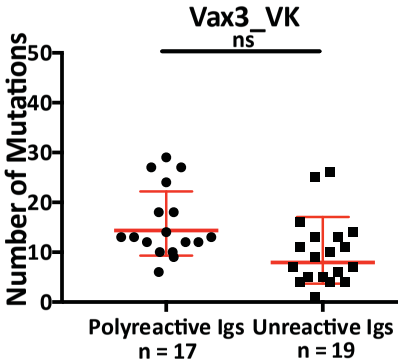

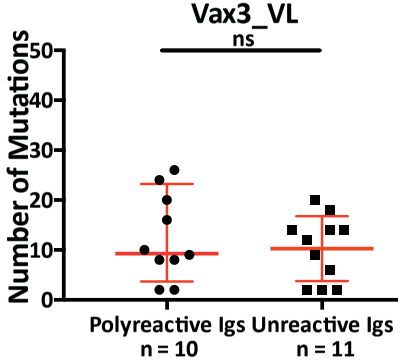

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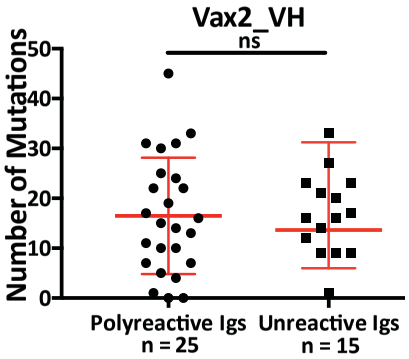

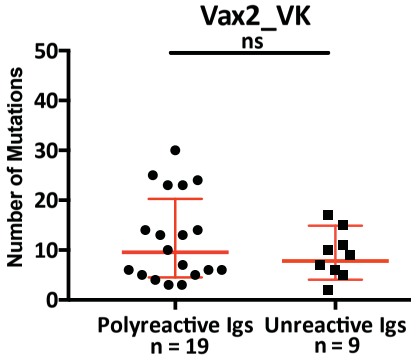

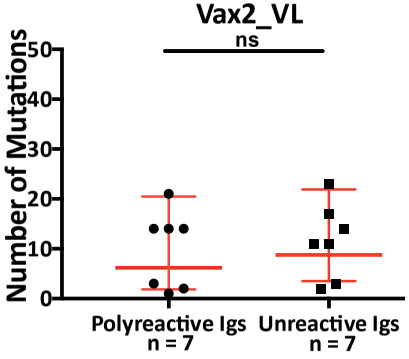

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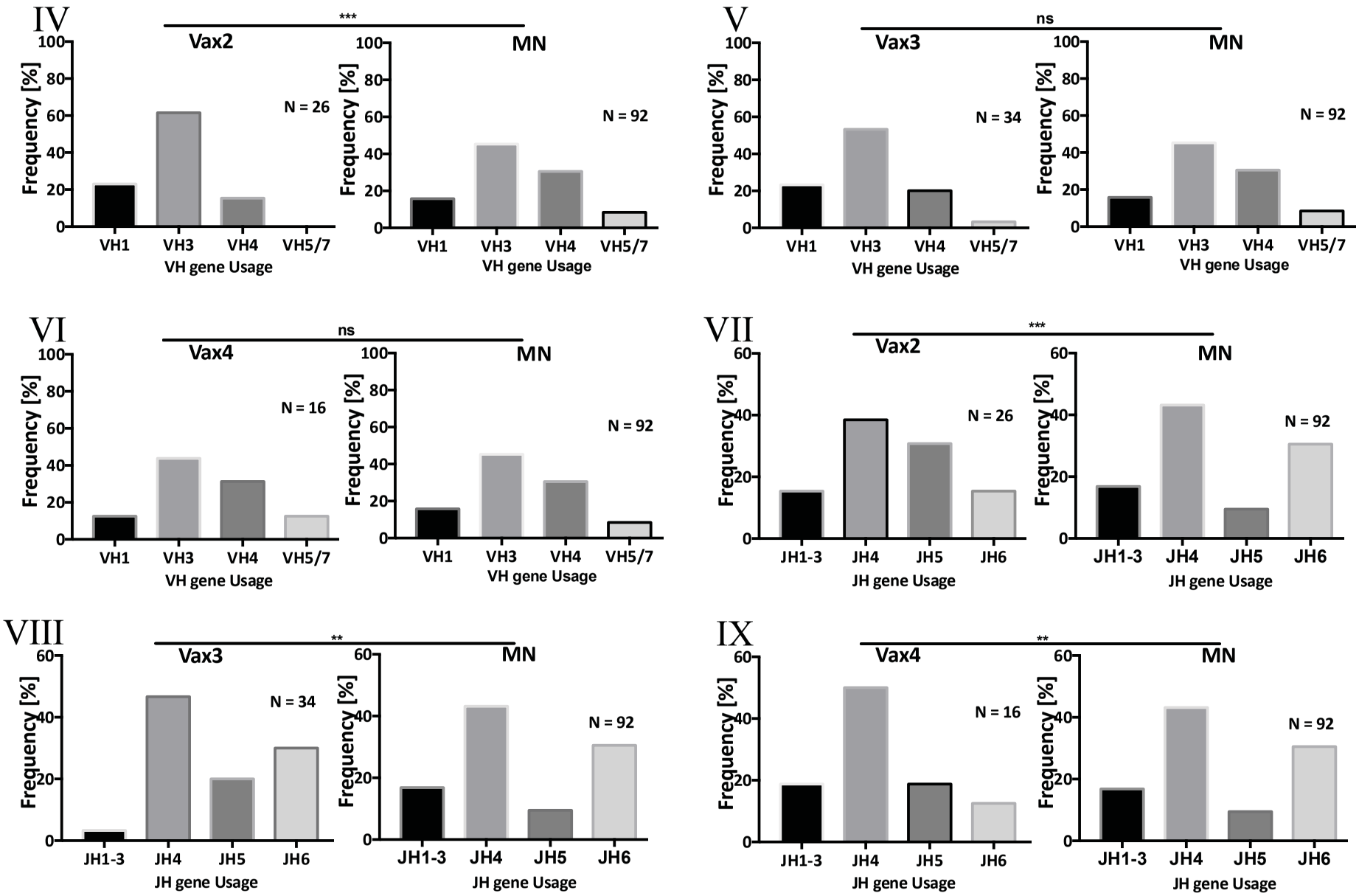
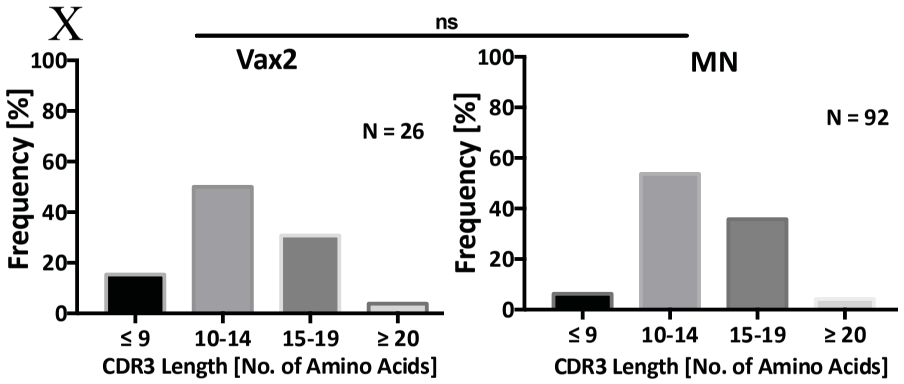
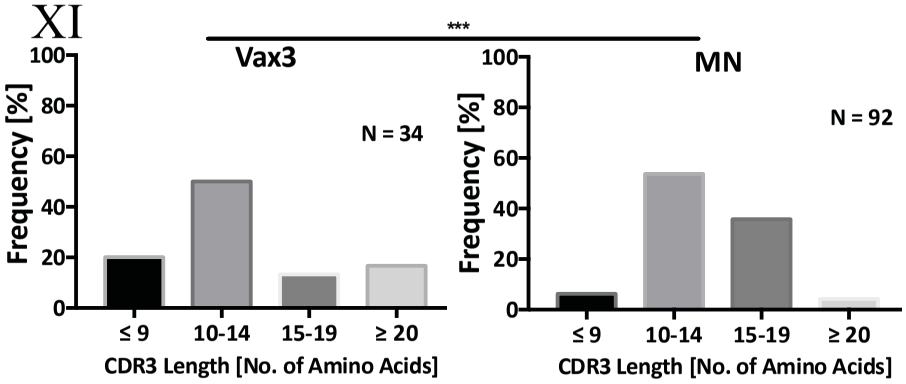
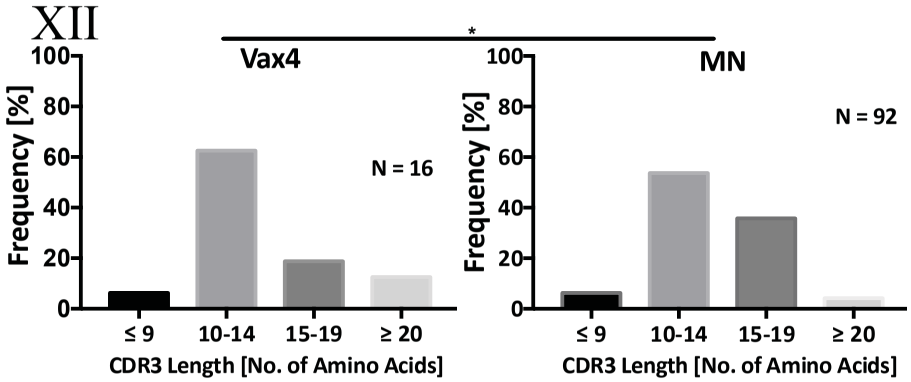

IV
***
Vax2
MN
Frequency [%]
N = 26
N = 92
VH1
VH3
VH4
VH5/7
VH gene Usage
V
ns
Vax3
N = 34
VI
Vax4
N = 16
VII
JH1-3
JH4
JH5
JH6
JH gene Usage
VIII
**
IX

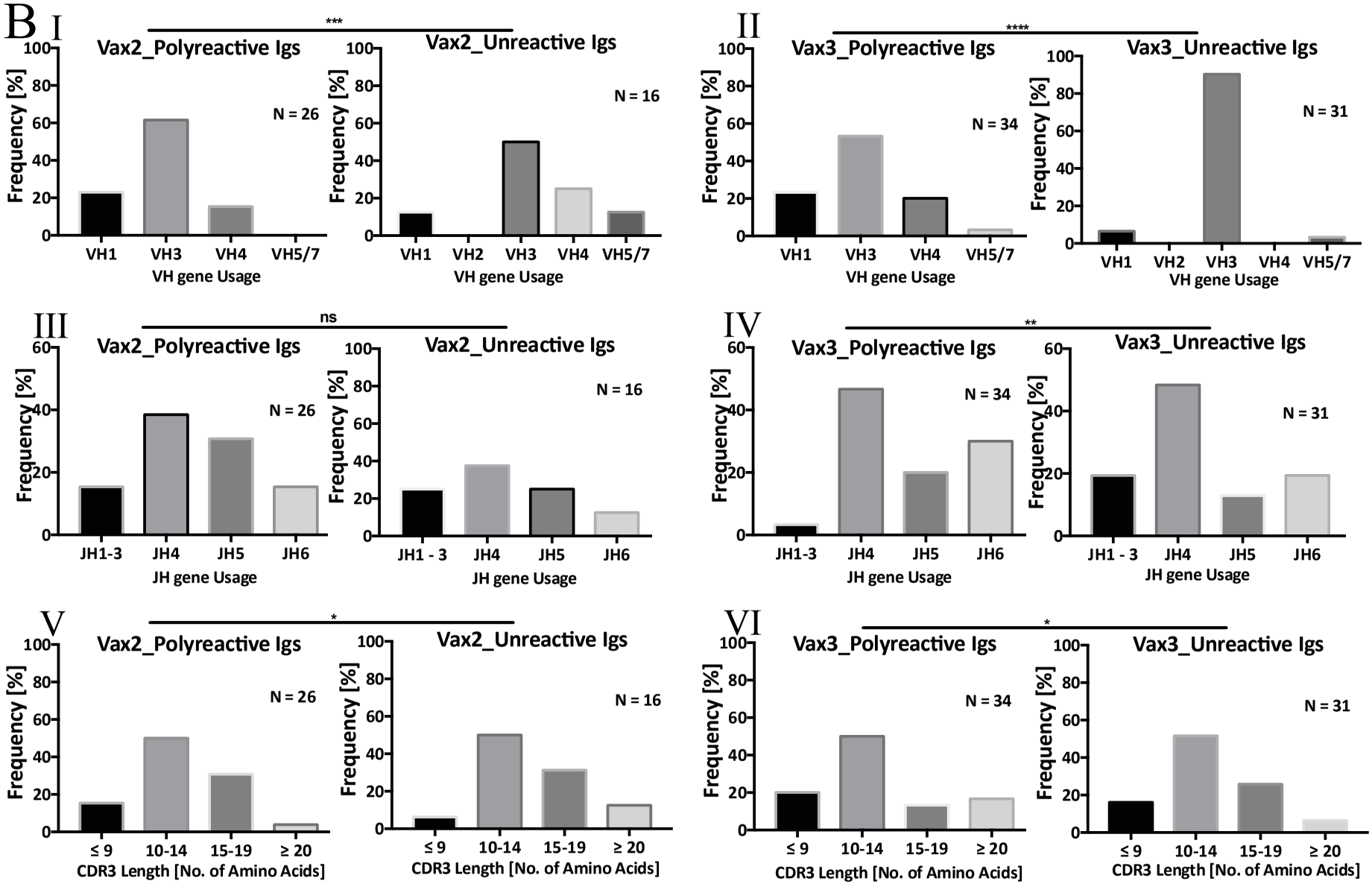
Continued


X
ns
Vax2
N = 26
MN
N = 92
Frequency [%]
100
80
60
40
20
0
≤ 9
10-14
15-19
≥ 20
CDR3 Length [No. of Amino Acids]


XI
***
Vax3
N = 34
MN
N = 92
Frequency [%]
100
80
60
40
20
0
≤ 9
10-14
15-19
≥ 20
CDR3 Length [No. of Amino Acids]


XII
*
Vax4
N = 16
MN
N = 92
Frequency [%]
100
80
60
40
20
0
≤ 9
10-14
15-19
≥ 20
CDR3 Length [No. of Amino Acids]

Continued


B
I
***
Vax2_Polyreactive Igs
Vax2_Unreactive Igs
Frequency [%]
N = 26
N = 16
VH1
VH2
VH3
VH4
VH5/7
VH gene Usage
II
****
Vax3_Polyreactive Igs
Vax3_Unreactive Igs
N = 34
N = 31
III
ns
JH1-3
JH1 - 3
JH4
JH5
JH6
JH gene Usage
IV
**
V
*
≤ 9
10-14
15-19
≥ 20
CDR3 Length [No. of Amino Acids]
VI

Continued

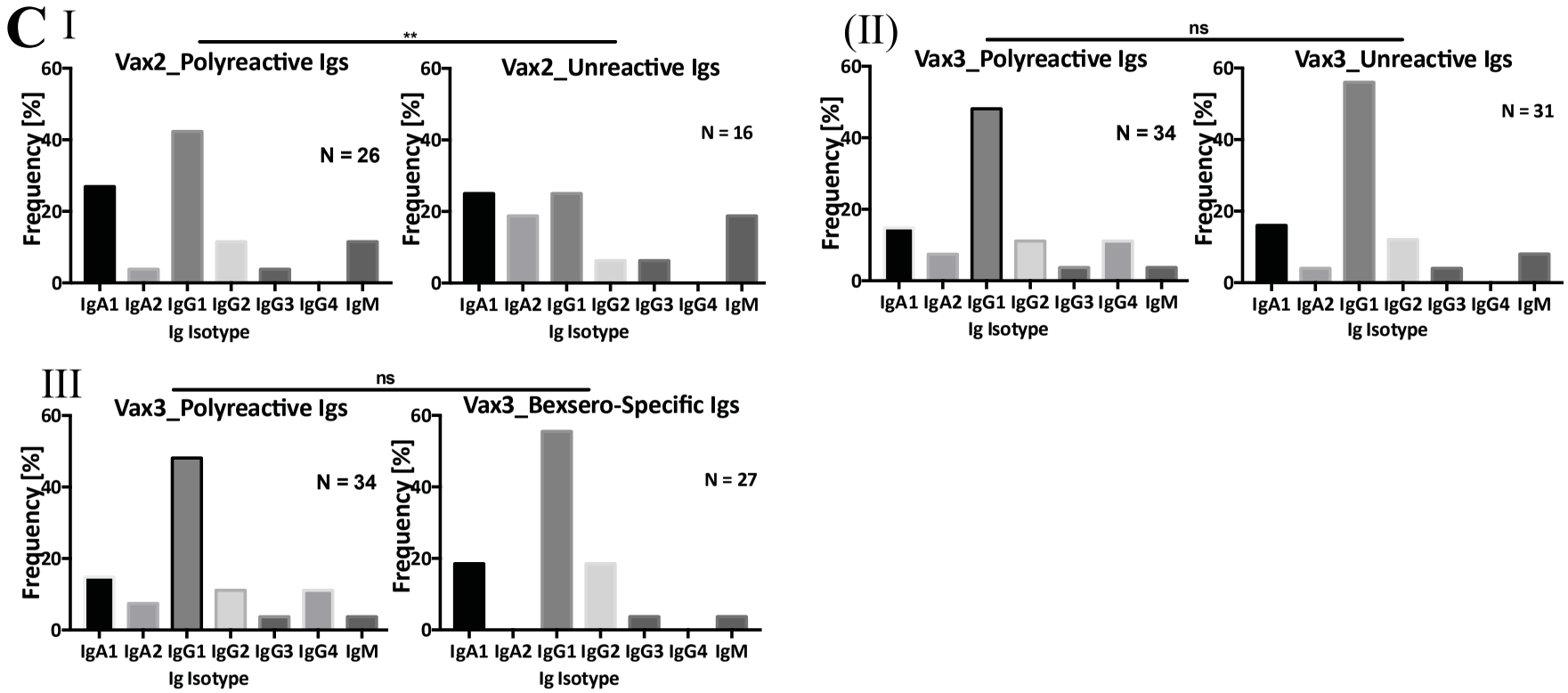


Fig. 12: Gene Analysis of polyreactive Vax2, Vax3 & Vax4 Igs: A (I) Vax3 SHM analysis of *IgVH*, *IgVk* and *IgVλ* of polyreactive Igs compared with Vax3 unreactive Igs. **(II)** Vax3 SHM analysis of *IgVH*, *IgVk* and *IgVλ* of polyreactive Igs compared with Vax3 Bexsero-specific-reactive Igs. **(III)** Vax2 SHM analysis of *IgVH*, *IgVk* and *IgVλ* of polyreactive Igs compared with Vax2 unreactive Igs. The geometric means and SD of the number of sequences analyzed are indicated. The p-values were calculated using student's t-test. **(IV)** Vax2 *IgVH* gene family usage pattern compared with the mature naïve population. **(V)** Vax3 *IgVH* gene selection pattern compared with the mature naïve population. **(VI)** Vax4 *IgVH* gene family usage pattern compared with the mature naïve population. **(VII)** Vax2 *IgJH* gene family usage pattern are compared with the mature naïve population. **(VIII)** Vax3 *IgJH* gene family usage pattern are compared with the mature naïve population. **(IX)** Vax4 *IgJH* gene family usage pattern are compared with the mature naïve population. **(X)** Vax2 CDR3 length usage pattern are compared with the mature naïve population. **(XI)** Vax3 CDR3 length usage pattern are compared with the mature naïve population. **(XII)** Vax4 CDR3 length selection pattern are compared with the mature naïve population. **B (I)** Vax2 *IgVH* gene family usage pattern compared with the Vax2 unreactive Ig population. **(II)** Vax3 *IgVH* gene family usage pattern compared with the Vax3 unreactive Ig population. **(III)** Vax2 *IgJH* gene family usage pattern compared with the Vax2 unreactive Ig population. **(IV)** Vax3 *IgJH* gene family usage pattern compared with the Vax3 unreactive Ig population. **(V)** Vax2 CDR3 length usage pattern are compared with the Vax2 unreactive Ig population. **(VI)** Vax3 CDR3 length usage pattern are compared with the Vax3 unreactive Ig population. **C (I)** Vax2 Ig isotype distribution compared with Vax2 unreactive Ig population **(II)** Vax3 Ig isotype distribution compared with the Vax3 unreactive Ig population **(III)** Vax3 Ig isotype distribution compared with Vax3 Bexsero-specific reactive Igs. **NB:** All frequencies were calculated as percentages of total Ig population. P-values were calculated using Fishers exact test.

Correlation between Ig gene features and Ig Bexsero reactivity properties

Vax2: Vax2 Igs reactive to Bexsero were mostly enriched in *IgVH3*, *IgJH4* Ig genes, IgG1 isotype, CDR3 length of 10 – 14 amino acids but with variability in SHM load {(fig. 13A(I)}. On the contrary, Vax2 Igs reactive to Bexsero rarely used *IgVH5/7*, Ig isotypes IgA2, IgG2 and IgG3, CDR3 lengths ≤ 9 or ≥20 {(fig. 13A(I)}. High affinity Vax2 Igs expressed IgG1 and IgG3 Ig isotypes. To further ascertain the relationship between Ig reactivity properties and Ig gene features, Vax2 polyreactive and unreactive Igs were analyzed separately. Vax2 polyreactive Igs were found to be enriched in *IgVH1*, *IgVH3*, *IgJH4* and *IgJH5* genes, variable SHM loads, variable CDR3 lengths and Ig isotypes (IgA1 and IgG1) {(fig. 13B(I)}. Unreactive Vax2 Igs were found to be enriched in *IgVH3*, *IgJH4* genes, variable SHM loads, CDR3 lengths of 10-14 or 15-19 amino acids and Ig isotypes (IgA1, IgA2 and IgG1) {(fig. 13B(II)}. Overall, Vax2 Igs reactive to Bexsero cannot be defined to a unique Ig gene profile.

Vax3: High binding Vax3 antibodies were be found to be enriched in *IgVH1, IgVH2*, *IgVH4* and *IgJH4* genes {(fig. 13A(II)}. These antibodies were mostly of the IgG1 isotype with CDR3 length between 10 – 14 amino acids and SHM ≤9 {(fig. 13A(II)}. *IgVH2* Igs uniquely selected only in the Bexsero-reactive Ig population belong in the high binding anti-Bexsero Ig population albeit with other high binding Igs selecting other *IgVH* genes. High binding Ig reactivity is therefore not a unique feature of Igs selecting *IgVH2*. Vax3 Igs reactive to Bexsero rarely showed any enrichment for *IgVH5/7*, Ig isotypes (IgA2, IgG2 and IgG3), CDR3 lengths ≤ 9 or CDR3 lengths ≥20 {(fig. 13A(II)}. Further analysis of Vax3 Bexsero-specific reactive Igs, unreactive Igs and Vax3 polyreactive Igs are shown in fig. 13B (III – V). Vax3 Bexsero-specific-reactive Igs are primarily enrich in *IgVH3*, *IgJH4*, SHM loads ≤ 9 or ≥ 20, CDR3 length of 10-14 amino acids and Ig isotype IgG1 {(fig. 13B(III)}. Nonetheless, these Igs are spread across all *IgVH*, *IgJH* genes, SHM loads, CDR3 lengths and all Ig isotypes but IgG4. Unreactive Vax3 Igs are also primarily enriched in *IgVH1* and *IgVH3* genes but diverse *IgJH* genes, SHM loads, CDR3 lengths and Ig isotypes without any unique selection pattern {(fig. 13B(IV)}. Vax3 polyreactive Igs also show no unique selection pattern in *IgVH* and *IgJH* gene selection, SHM loads, CDR3 length distribution or Ig isotypes {(fig. 13B(IV)}. Overall, Vax2 Igs reactive to Bexsero cannot be defined to a unique Ig gene profile.

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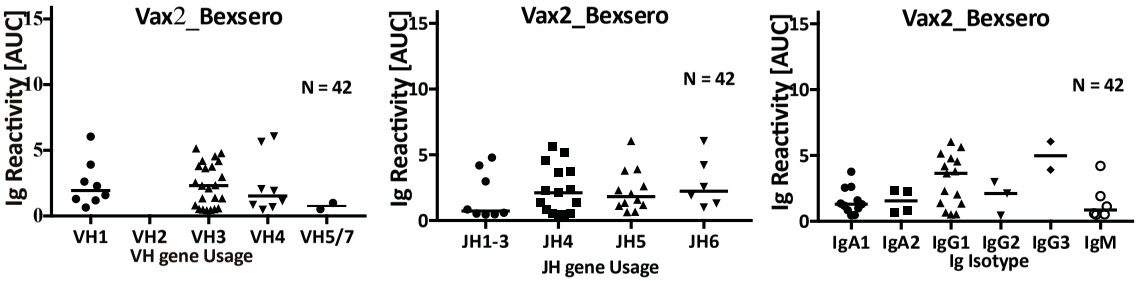

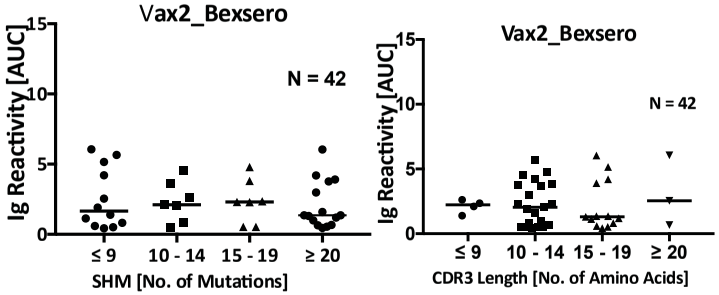

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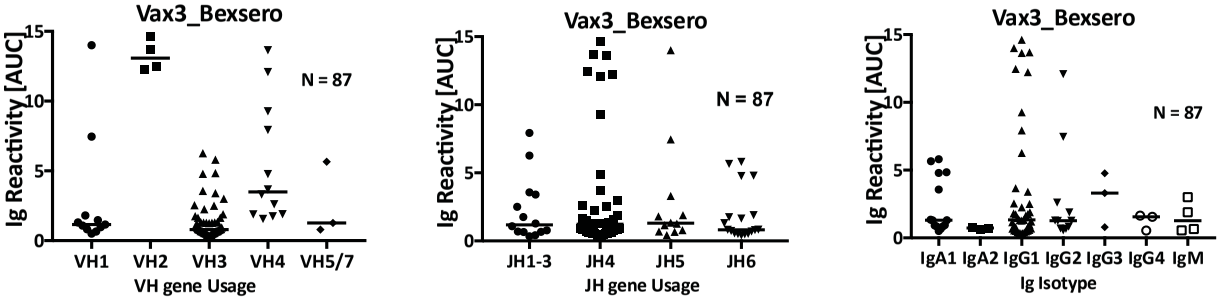

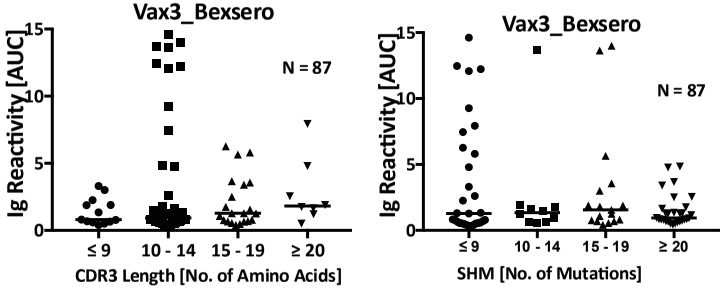

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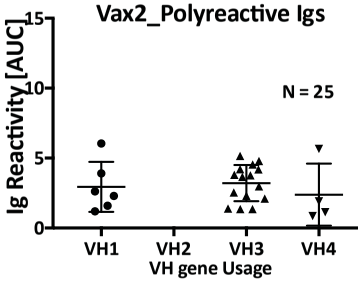

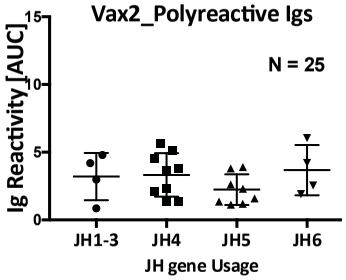

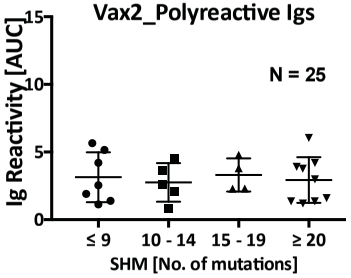

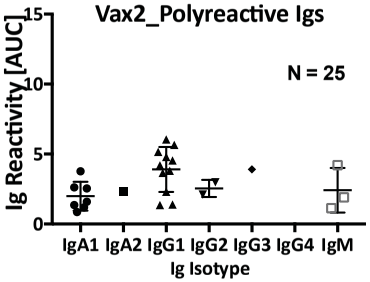

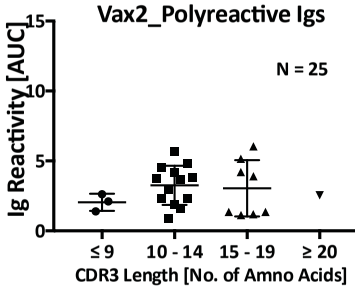

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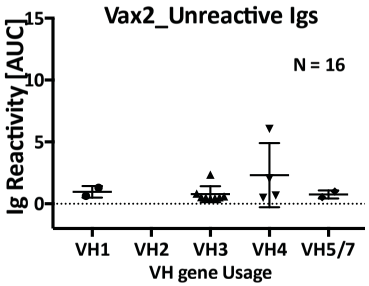

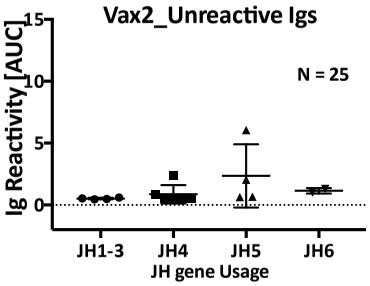

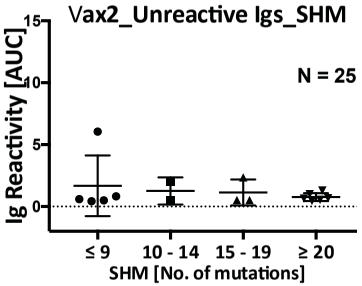

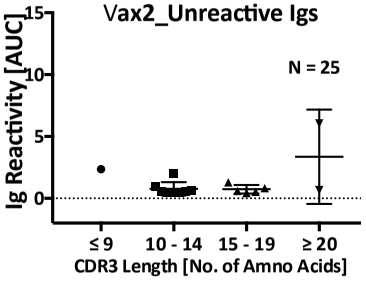

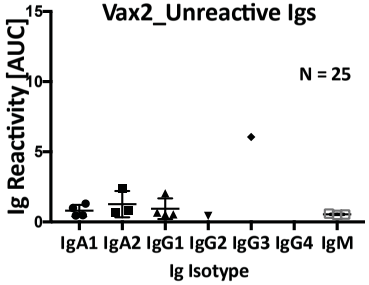

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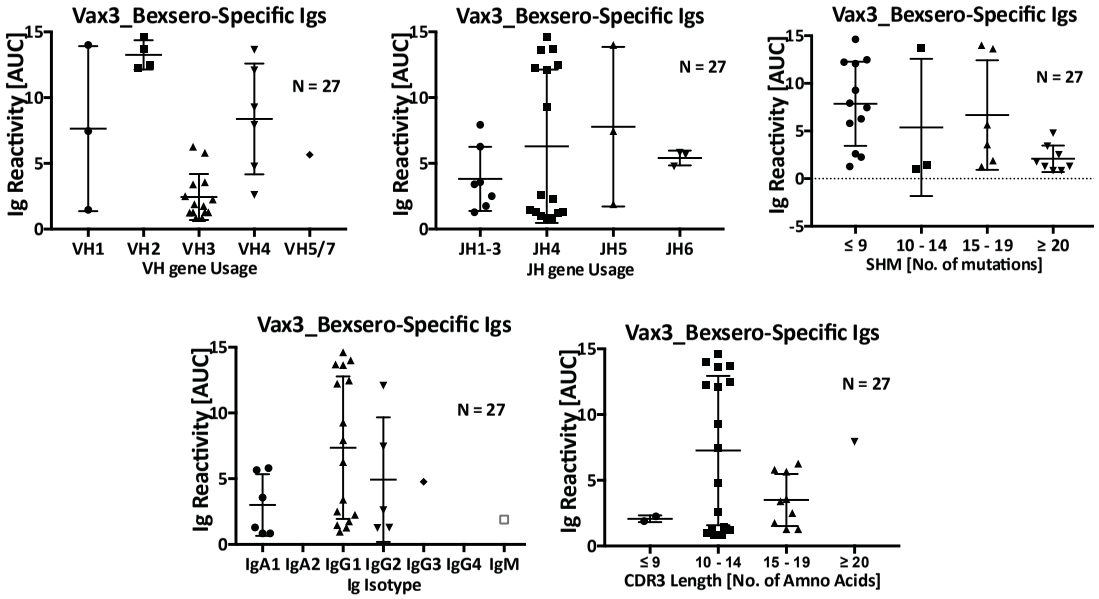

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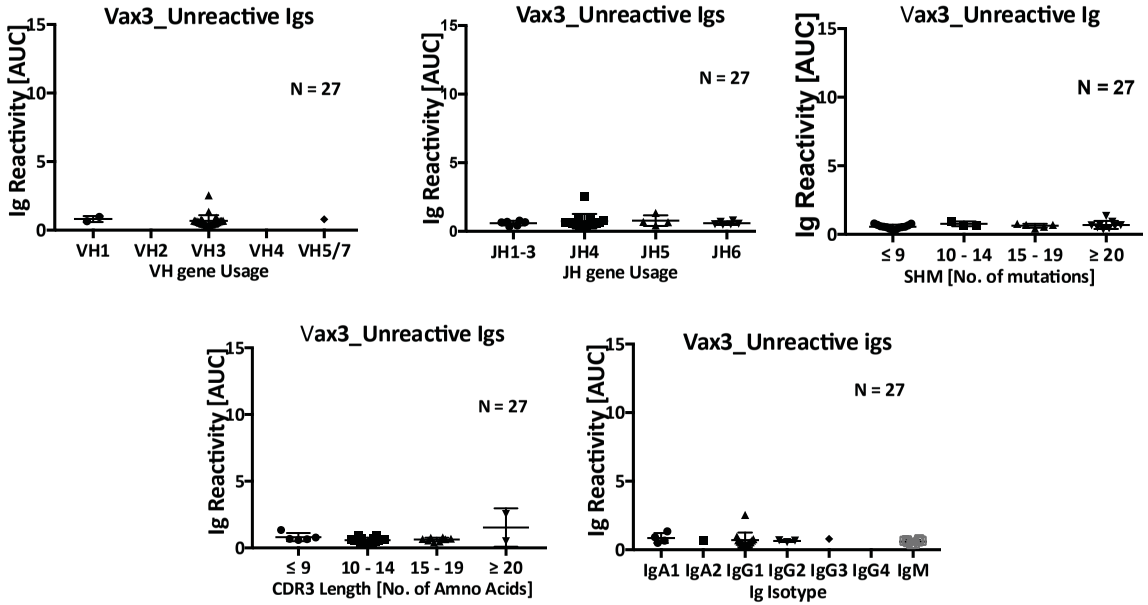

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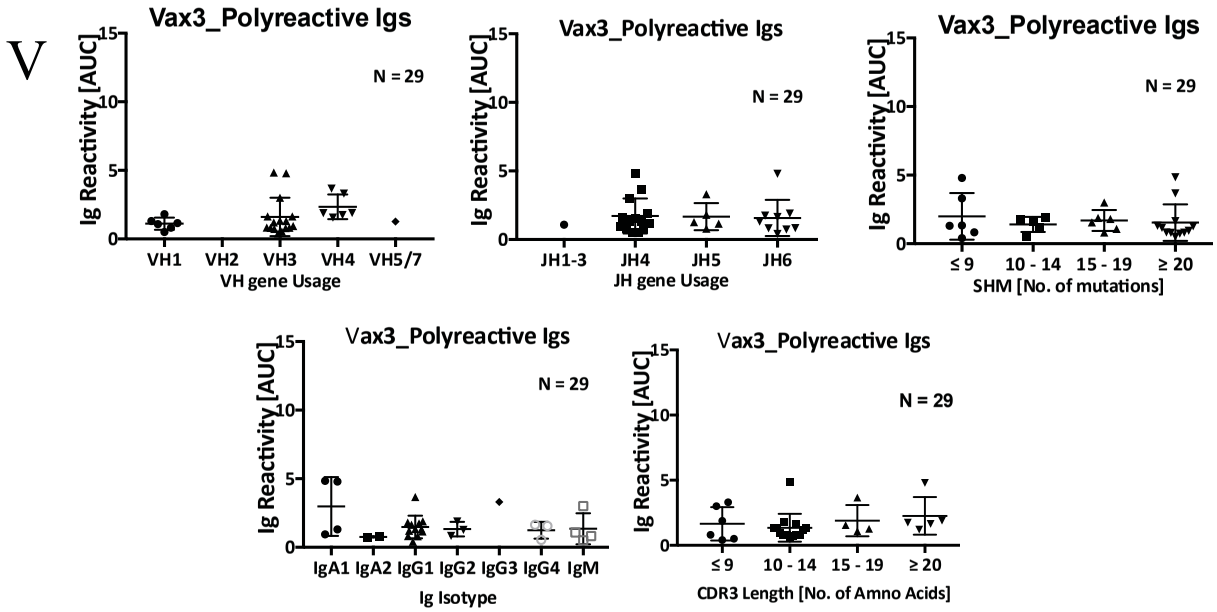

Fig. 13: A. Correlation between Ig gene features and Ig reactivity properties. (I) Vax2 Igs: correlation between *IgVH* and *IgJH* gene family usage, CDR3 length, SHM load and Ig isotype distribution with Ig reactivity to Bexsero (**II**) **Vax3 Igs:** correlation between *IgVH* and *IgJH* gene family usage, CDR3 length, SHM load and Ig isotype distribution with Ig reactivity to Bexsero. AUC values were calculated from the average of three separate ELISA experiments. **B. Correlation between Ig gene features and Ig reactivity properties in different Ig subsets of Vax2, Vax3 Igs (I) Vax2 polyreactive Igs:** correlation between *IgVH* and *IgJH* gene family usage, CDR3 length, SHM load and Ig isotype distribution with Ig reactivity to Bexsero (**II**) **Vax2 unreactive Igs:** correlation between *IgVH* and *IgJH* gene family usage, CDR3 length, SHM load and Ig isotype distribution with Ig reactivity to Bexsero (**III**) **Vax3 Bexsero - reactive Igs:** correlation between *IgVH* and *IgJH* gene family usage, CDR3 length, SHM load and Ig isotype distribution with Ig reactivity to Bexsero (IV) **Vax3 Bexsero - unreactive Igs:** correlation between *IgVH* and *IgJH* gene family usage, CDR3 length, SHM load and Ig isotype distribution with Ig reactivity to Bexsero (**V**) **Vax3 Bexsero - polyreactive Igs:** correlation between *IgVH* and *IgJH* gene family usage, CDR3 length, SHM load and Ig isotype distribution with Ig reactivity to Bexsero. AUC values were calculated from the average of three separate ELISA experiments.

Bexsero-Specific Reactive Ig Binding to *Neisseria meningitidis* MC5 in Vitro

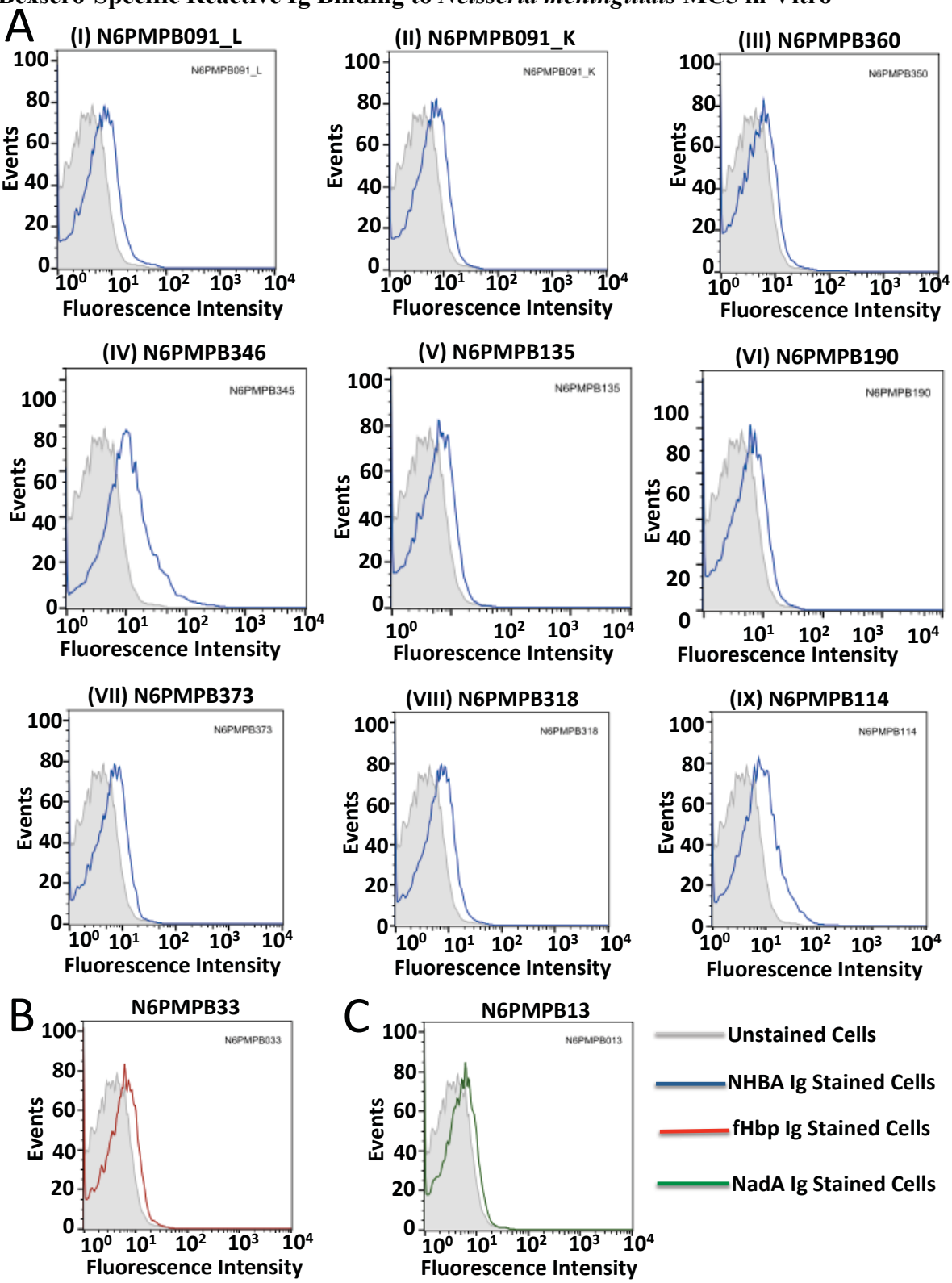

Fig. 14: Binding Affinity of monoclonal Bexsero-Specific-Reactive Igs to *Neisseria meningitidis* mc58 in vitro, measured by flow cytometry. A: Plots of affinity profiles of NHBA antibodies **B:** Plots of affinity profiles of fHbp antibodies **C:** Plots of affinity profiles of NadA antibodies.

Monoclonal antibodies generated against recombinant NHBA, NadA and fHbp were tested on endogenously expressed NHBA, NadA and fHbp on *Neisseria meningitidis* mc58 at 10 ug/mL antibody concentrations in FACS. Four anti-NHBA antibodies selected from three different clusters (N6PMPB091_K and N6PMPB091_L: cluster 1| N6PMPB373: cluster 2| N6PMPB114: cluster 5) all bind weakly to endogenous NHBA despite binding either moderately (N6PMPB114 and N6PMPB091_L) or strongly (N6PMPB091_K and N6PMPB373) to recombinant NHBA in ELISA (fig. 14A). Five non-cluster anti-NHBA antibodies (N6PMPB350, N6PMPB318, N6PMPB345, N6PMPB135 and N6PMPB190) also all bind weakly to the endogenous NHBA despite N6PMPB345 binding moderately to recombinant NHBA although N6PMPB350, N6PMPB318, N6PMPB135 and N6PMPB190 also bind weakly to the recombinant NHBA in ELISA (fig. 14A). One each, of the anti-fHbp (N6PMPB033: fig. 14B) and anti-NadA (N6PMPB013: fig. 14C) antibodies which both bind moderately to recombinant fHbp and NadA also bind weakly to the endogenously expressed fHbp and NadA respectively. (This data was obtained from experiments carried out by Prof. Christoph Tang at the William Dunn School of Pathology at Oxford University)

Summary of Vaccination Scheme, Serum and Plasmablast Data

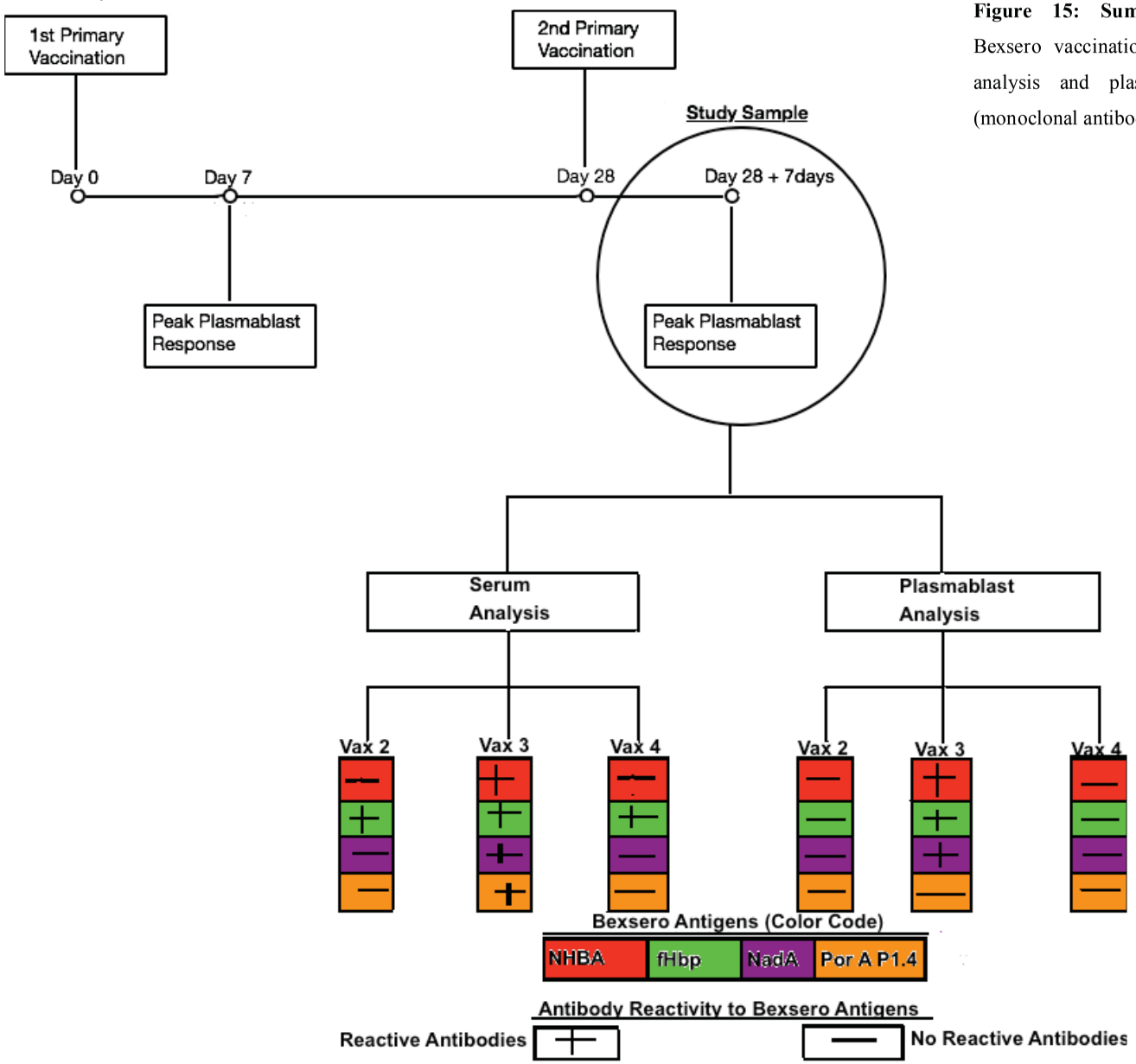

Figure 15: Summary: Scheme integrating Bexsero vaccination plan, Serum Ig reactivity analysis and plasmablast reactivity analysis (monoclonal antibodies).

Table 1: Summary of Vax2, Vax3 and Vax4 Plasmablast analysis

	Vaccinee		
Parameter	**Vax2**	**Vax3**	**Vax4**
Plasmablast freq. [%]	0.46	3.76	0.43
Freq. of NHBA-GNA2091 Antibodies [%]	0	24.2	0
Freq. of fHbp-GNA1030 Antibodies [%]	0	2.2	0
Freq. of NadA Antibodies [%]	0	2.2	0
Freq. of PorA P1.4 Antibodies [%]	0	0	0
Freq. of Polyreactive Antibodies [%]	62	33	89
Freq. of Unreactive Antibodies [%]	38	37.4	11

Blood samples from 7 days post second primary vaccination were established as study samples. Vax2 showed serum Igs against fHbp-GNA1030, plasmablast frequency of 0.46% and no reactive monoclonal Igs against any of the Bexsero antigens (NHBA-GNA2091, fHbp-GNA1030, NadA and PorA P1.4). Vax3 showed serum Igs against NHBA-GNA2091, fHbp-GNA1030, NadA and PorA P1.4, plasmablast frequency of 3.76%, twenty-two monoclonal antibodies against NHBA-GNA2091, two monoclonal antibodies against fHbp-GNA1030 and two monoclonal antibodies against NadA. Vax4 showed serum Igs against fHbp-GNA1030, plasmablast frequency of 0.43% and no reactive monoclonal Igs against any of the Bexsero antigens (NHBA-GNA2091, fHbp-GNA1030, NadA and PorA P1.4). The lack of monoclonal antibodies against any of the Bexsero antigens in Vax2 and Vax4 fails to correlate the strong anti-fHbp-GNA1030 antibody response observed in sera of both vaccinees. The quality of the Bexsero response in Vax2 and Vax4 with plasmablast frequencies (Vax2: 0.46% and Vax4: 0.43%) post-second primary vaccination therefore raises questions. All Bexsero-reactive monoclonal Igs generated from plasmablast of Vax2 and Vax4 are polyreactive Igs aspecific to Bexsero. The frequency (2/26) and Bexsero-reactivity (AUC values) of anti-fHbp-GNA1030 antibodies also fail to correlate with the strong anti-fHbp-GNA1030 antibody response observed in serum of Vax3. The frequency (2/26) and Bexsero-reactivity (AUC values) of anti-NadA antibodies appear to reflect the serum anti-NadA antibodies, detected albeit weakly in serum of Vax3. The frequency of anti-NHBA antibodies (22/26) strongly correlates with the serum anti-NHBA antibody response. The antibody response to Bexsero in Vax3 appears to be predominantly targeted to NHBA and suggests that serum anti-fHbp-GNA1030 antibodies detected in Vax3 may not have come from the second primary vaccination. At sampling frequencies of Vax3 [Bexsero reactive antibodies = 6.8% (26/384) and polyreactive antibodies = 6.8% (26/384)], deeper sampling of the PBMCs of Vax3 would yield more anti-NHBA-GNA2091, anti-fHbp-GNA1030 and anti-NadA antibodies and potentially some anti-PorA p1.4 antibodies. On the

contrary, at the sampling frequencies of Vax2 [Bexsero reactive antibodies = 0% and polyreactive antibodies = 13.5% (26/192)] and Vax4 [Bexsero reactive antibodies = 0% and polyreactive antibodies = 16.7% (16/96)] deeper sampling may potentially yield some anti-NHBA-GNA2091, anti-fHbp-GNA1030, anti-NadA anti-PorA p1.4 antibodies. This data therefore is overall conclusive of the B cell response to Bexsero in Vax2, Vax3 and Vax4. In conclusion, it appears highly probable that anti-fHbp-GNA2091 antibodies detected in sera of Vax2, Vax3 and Vax4 must have come from response to the first primary vaccination. The response to the second primary vaccination largely recruited polyreactive antibodies in all three vaccinees. Vax3 also predominantly targeted NHBA-GNA1030 in the second primary vaccination.

Chapter 3: Discussion

Vaccination and Serum Anti-Bexsero Antibody Response

Bexsero clinical trials by Novartis, established the generation of bactericidal antibodies against the main antigenic components (NadA, fHbp-GNA2091, NHBA-GNA1031 and Outer membrane vesicle (OMV - with PorA P1.4 as main antigen)) in infants, children, adolescents and adults vaccinated with Bexsero, albeit from varying doses detectable at titers $\geq$ 1:4 [42]. The three individuals recruited to participate in this project were administered with two doses of Bexsero at a one-month interval between doses, in accordance with the Novartis recommended dosage for adults. To assess the human B cell response post-vaccination, plasmablast was established as the target compartment within the antigen-experienced B cell pool, to study. Plasmablasts are rapidly dividing pre-plasma cell intermediaries differentiated from germinal center B cells. The plasmablast compartment was targeted because alternative B cell compartments like plasma cells home to the bone marrow (difficult to access) and memory B cells generally have lower binding affinities relative to plasmablast [23,52].

The plasmablast frequency peaks at 6 or 7-days post-vaccination [43]. The experimental design established blood samples from the Vaccinees at day 0 (prior to first primary vaccination) and day 28 (prior to second primary vaccination) as baseline samples for assessing the plasmablast response at 7 days post first and post second primary vaccinations respectively. Differences in antibody repertoire between the first and second primary vaccinations and further downstream questions on repertoire differences could be answered by comparing the characteristics of the sera and plasmablast populations between the two vaccination time points. In the absence of plasmablast and sera from the first primary vaccination, the impact of the first primary response could only be observed through "genetic footprints" (Ig sequences) or protein markers (serum antibodies) observable in the B cell response to the second primary vaccination. The antibody repertoire generated in response to the second primary vaccination can be categorized into two populations ("newly recruited B cell pool" or "memory B cell pool from first primary vaccination"). Secreted antibodies from the first primary response in sera may also impact the quality of antibody response to individual antigens in the second primary vaccination through "epitope masking". "Epitope masking" is a negative feedback mechanism exploited during B cell responses to abrogate germinal center reactions through masking of epitopes of antigens presented in the germinal center by pre-existing serum antibodies, concurrently preventing further engagement of antigens by newly recruited mature naïve B cells or memory B cells [53]. Despite the outlined limitations due to the unavailability of the samples from the first primary vaccination, the available samples

from the second primary vaccination provided enough material to attempt answering the questions outlined in this project.

Plasmablast intermediates differentiate into short-lived plasma cells, which secrete antibodies at a higher rate for only a few weeks or long-lived plasma cells, which home into the bone marrow and secrete antibodies for years [22–24]. Antibodies detectable primarily in sera provide an immediate verification marker for a qualitative assessment of the immune response post-vaccination. Data from serum ELISA against Bexsero indicated strong anti-Bexsero antibody response in all three vaccinees (Vax2, Vax3 and Vax4). On the contrary, sera from all three vaccinees failed to react to two of the randomly sampled structurally diverse antigens (insulin and dsDNA) albeit weakly to LPS. However, LPS is a common bacterial antigen readily exposed to the human immune system upon exposure to pathogenic and non-pathogenic bacteria, hence the high likelihood of randomly selected individuals possessing anti-LPS antibodies in sera. The combined data establishes the observed serum antibody reactivity to Bexsero, as resulting from a specific interaction between anti-Bexsero antibodies and Bexsero antigens. The predominant IgG response mirrors the data reported in the Bexsero clinical trial report by Novartis [34], and also suggest expected germinal center reaction (class-switch recombination) and a non-mucosal B cell response. The subsequent western blot experiment was conducted to ascertain the constituents of the serum anti-Bexsero antibody pool. Western blot analysis of serum Igs at titer 1:200 detects anti-fHbp-GNA2091 antibodies in all three vaccinees and anti-NHBA-GNA1030, anti-PorA P1.4 and anti-NadA antibodies in Vax3 only. Novartis Bexsero clinical trials, reports human serum bactericidal activity at titers ≥ 1:4 against all four Bexsero antigens (NadA, fHbp-GNA2091, NHBA-GNA1030 and PorA P1.4) post vaccination [34] but the ELISA and western blot technique used to detect anti-Bexsero antibodies measure unspecific antibody binding at titers higher than 1:200 hence presents a difficulty in determining if Vax2 and Vax4 posses serum anti-NHBA-GNA1030, anti-NadA and anti-PorA detectable at high titers. The serum anti-Bexsero antibody pool therefore appears dominated by anti- fHbp-GNA2091 antibodies in all three vaccinees for unclear reasons. Beernink et al, 2015 have also reported the detection of serum bactericidal antibodies against OMV antigens (particularly Por A P1.4), fHbp-GNA2091 and NHBA-GNA1030 post-Bexsero vaccination, further supporting the observations made in the Novartis clinical trial report [54]. fHbp-GNA2091 may be the most immunodominant of the Bexsero antigen constituents selectively targeted in the immune response. Immunodominance has been established to selectively focus the immune response on a few of many potential epitopes upon exposure to pathogens that present large numbers of epitopes on multiple

antigens to the host immune system [11]. Differences in antigen immunodominance may result from variability in the accessibility of binding sites on the antigen, genetic diversity of the host's naïve B cell population, stochastic founding events in germinal center, genetic predisposition mediated by helper T cells and chance mutations during affinity maturation [16]. Alternatively, the vaccinees may have reacted poorly to the other Bexsero antigens. The two main potential reasons for vaccine failure are i. failure of the vaccine delivery system to properly deliver vaccines and ii. failure of the immune response due to inadequacies of the vaccine or inherent host factors [55]. It appears highly plausible that a myriad of factors may have forced selectivity in the immune response to the different Bexsero antigens. Lastly, the frequency of antibody secreting plasma cells specific to the other Bexsero antigens may have been too low or the affinity of antibodies against other Bexsero antigens may have been too weak to detect serum antibodies at titer 1:200. All these possibilities raise questions about the fine specificities of the antibody repertoire generated in response to multi-antigen complexes.

Immensely Expanded Ig Diversity in B Cell Response to Bexsero

The reason(s) for the conspicuous difference in plasmablast frequencies between Vax3 (3.76%), Vax2 (0.46%) and Vax4 (0.43%) is unclear. Inherent host factors of vaccinees may have interacted differently with the vaccine [55], resulting in a weak plasmablast response in Vax2 and Vax4. This speculation partially contradicts Vax2 and Vax4 serum reactivity data in ELISA and western blot, both of which indicate a strong anti-Bexsero antibody and specifically anti-fHbp-GNA1030 antibody response respectively, post Bexsero vaccination. Alternatively, the peak plasmablast response time may have been missed in both Vax2 and Vax4, prior to plasmablast isolation. The samples were taken 7-days after the second primary vaccination. The plasmablast frequency peaks at 6 or 7-days post-vaccination [43]. Wrammert et al, 2008 have shown in the human B cell response to influenza virus that, the antibody-secreting cell response is quite transient, peaking at approximately day-7 and returning to barely detectable levels at day-14 post-vaccination [56]. Due to prior exposure of the vaccinees to Bexsero, one month before the second vaccination, the recall of memory B cells specific to Bexsero from the first vaccination may have altered the response time for the second plasmablast response leading to an earlier peak plasmablast response time point. However newly recruited mature naïve B cells require 6 to 7 days to differentiate into plasmablast upon exposure to vaccine antigens. Missing the early secondary response driven primarily by memory B cell population from the first primary vaccination is unlikely to significantly impact the peak plasmablast response time, post second primary vaccination. It has however

been established that antibodies from response to the first primary vaccination and early second primary vaccination impact affinity maturation in subsequent vaccinations by binding to newly introduced antigens and divert them to phagocytes for degradation and disposal [11]. Sufficient pre-existing antibodies can clear the antigen(s) and prevent induction of immune response to the antigen(s) [11]. This may account for the poor plasmablast response observed in Vax2 and Vax4 but contradicts the observation made in Vax3. Thomas Keppler, PhD in his unpublished work presented at Keystone symposia SciTalks 2017 on B cell clonal dynamics during sequential immunizations, showed data indicating a dip in total plasmablast frequency after the fourth immunization, also suggesting a likely consequence of a biological phenomenon [unpublished]. Andrews et al, 2015 have also shown that high pre-existing serological antibody levels to a given influenza virus strain correlate with low production of antibody-secreting cells and memory B cells recognizing that strain upon re-vaccination [57]. These alternate findings give credence to the observed weak plasmablast frequencies in Vax2 and Vax4 as a consequence of a biological phenomenon and not an artifact due to errors in the experimental procedure.

Ig Gene Analysis: The Ig genes of the mature naïve B cells provide a base line to access for skews or unique selection patterns in the antigen-experienced Ig population of Vax2, Vax3 and Vax4.

SHM & Class Switch: Plasmablast populations from Vax2, Vax3 and Vax4 all indicated affinity maturation with distributions of weakly mutated, moderately mutated and highly mutated antibodies that are also mostly class switched. Affinity maturation is an important event in germinal center reaction, during which somatic hypermutation of V-regions of B cells and clonal selection evolutionarily force selection for survival of B cells with high affinity for the antigen(s) [11]. VH, VK or VL genes are not significantly more or less mutated respectively between Vax2, Vax3 and Vax4, suggesting a response to a common antigen(s) albeit, the accumulated mutations and the class switched nature of the Ig population have no predictive potential of the affinity and functional quality of the Ig response. Antibodies generated in humans in response to *Neisseria meningitidis* infection primarily participate in antibody-mediated complement activation [58]. IgG and IgM isotypes are capable of inducing the classical complement pathway for subsequent bactericidal opsonization or bacterial lysis [11]. IgA antibodies which make up 25% - 40% of total Igs in all Vaccinees, have been implicated in the activation of the manan-binding lectin of complement activation in sera although IgA are functionally more potent when homed to mucosal surfaces to attach and prevent bacteria from penetrating epithelial surfaces [11,59,60]. Regardless, IgG1, which is the

most predominant Ig in the blood, predominates the Ig distribution in all the vaccinees as observed in sera. The data does not point to any deliberate evolutionary selection for more potent complement-activating Ig isotypes. It appears to be a product of a statistical correlation with the natural propensity for expression of each of the Ig isotypes but overall with favorable capacity for complement activation.

VH, JH & CDR3 Length: Usage patterns in the Ig heavy chain variable region (*IgVH*) and joining region (*IgJH*) genes in all three vaccinees appear to indicate significant equilibrium shift in frequency distribution of classically selected Ig (VH1, VH2, VH3, VH4, VH5 and VH6) and Ig (JH1, JH2, JH3, JH4, JH5 and JH6) genes when compared to the mature naïve Ig population. The differential changes in selection are significant in Vax3 Ig (VH2 and VH3) and Vax2 Ig (JH (1-3), JH5 and JH6) and Vax4 Ig (JH (1-3) and JH6), generally indicating clear changes in the *IgVH* and *IgJH* frequency distribution upon exposure to Bexsero antigens. On the contrary, no selection pattern or skew is observed in the CDR3 length distribution between the Igs of the vaccinees and the mature naïve Igs. Trück et al, 2015 have also shown in vaccination with a conjugate vaccine containing *Haemophilus influenza* type b (Hib) and group C meningococcal polysaccharides, as well as tetanus toxoid (TT), that CDR3 length distributions of all Ig isotypes were similar between isotypes before and after vaccination [61]. Regardless, there is an overall clear distinction in the Ig gene repertoire between antigen experienced Bexsero Vaccinees plasmablast and mature naïve B cells.

Clone/Cluster Igs: The Ig genes were further analyzed for clonal selection. On binding to an antigen, B cells are activated to divide and produce many identical progeny called clones [11]. Clones are exact genetic copies of the progenitor B cell. During affinity maturation, somatic hypermutation introduces mutations primarily in the Ig(V) genes, accumulating often numerous amino acid changes in the antibody CDR3 region. The random nature of somatic hypermutation introduces minor genetic diversity in the clonal pool with often differences in B cell receptor affinity within the clonal pool. These new groups of related B cells are called cluster antibodies. Cluster antibodies originate from a common Ig progenitor, sharing the same heavy chain, light chain, isotypes, CDR3 length and same/similar CDR3 composition and number of somatic hypermutations. On the contrary, antibody "families" defined in table 18 include antibodies that appear to originate from a common Ig lineage or converge from different Ig lineages as determined by their Ig gene features. Convergence clonality suggests specific signatures of Ig gene rearrangement [62] which could facilitate surveillance in immunological memory at community level [63]. In the event of memory B cell reactivation, B

cells of the same clone or cluster may be recruited into different germinal centers leading to variation in somatic hypermutation and class switch recombination [47]. Such populations of B cells may also appear to converge from different Ig lineages despite commonality in original Ig progenitor. Both Vax3 and Vax4 exhibit clonal expansion, although relatively poorly but further establishing the antigen-experienced status of the Ig populations specifically for Vax3 and Vax4. Wang et al, 2015 showed through in silico simulation that affinity maturation can be frustrated by conflicting selection forces imposed by the different antigens of a multi-antigen complex [64]. This may explain the poor clonal expansion in the B cell populations of all three vaccinees in response to Bexsero. In conclusion, the Igs of Vax2, Vax3 and Vax4 all possess markers pointing to antigen experience, albeit, strongly in the Vax3 population, and indicate a immensely diverse Ig population with unique selection patterns in isotype distribution, *IgVH* and *IgJH* gene repertoires, CDR3 length distribution and clonal selection within vaccinees Ig populations and between vaccinees Ig populations.

Anti-Bexsero Monoclonal Antibody Reactivity Properties

The variation in the quality of the anti-Bexsero antibody response between Vax2, Vax3 and Vax4 observed in the plasmablast FACS data appears to also reflect in the data on monoclonal antibody reactivity to Bexsero. Vax3 [plasmablast frequency: 3.76%] showed a distribution of highly reactive Igs, moderately reactive Igs, weakly reactive Igs and unreactive Igs compared to Vax2 [plasmablast frequency: 0.46%] and Vax4 [plasmablast frequency: 0.43%] both of which showed distributions of moderately reactive Igs, poorly reactive Igs and unreactive Igs. The distribution also showed antibody panels from Vax2, Vax3 and Vax4 that cross-react with structurally diverse self antigen(s) (insulin and dsDNA) and non-self antigen(s) (LPS) in addition to Bexsero. These Igs appear to be polyreactive Igs aspecific to any of the Bexsero antigens, recruited into the response to the second primary vaccination. Polyreactive Igs have been shown to be a major driver in the broad antibacterial activity of the natural Ig repertoire [65]. Polyreactive Igs lyse bacteria and enhance phagocytosis in the presence of complement [65]. They also bind to apoptotic cells and with complement, enhance the phagocytosis of these cells by macrophages [65]. The lack of Bexsero specific Igs in the Vax2 and Vax4 Ig population reinforces the hypothesis that Vax2 and Vax4 responded poorly to the vaccine and raises questions about the origin of the strong anti-fHbp-GNA1030 Igs observed in sera of the two vaccinees. Serum anti-fHbp-GNA1030 Igs observed in sera of Vax2 and Vax4 must have originated from the response to the first primary vaccination. Although serum Igs against NHBA-GNA2091 was only detectable in Vax3 but not Vax2 and Vax4, as well as serum Igs against NadA and OMV antigens are

detectable albeit very weakly in Vax3 but undetectable in Vax2 and Vax4 at titer 1:200, Novartis reports serum bactericidal activity at titer ≥ 1:4. There is a possibility for pre-existing serum antibodies to abrogate the induction of B - cell response to the same antigen, upon re-exposure through epitope masking to prevent further engagement by B cells or formation of antibody:antigen complexes to be cleared by phagocytes [11,53]. However, this hypothesis is unlikely to apply to NHBA-GNA2091, NadA and OMV due to the potentially low titers in Vax2 and Vax4, if any at all. Serum antibody restriction of the B cell response against fHbp-GNA1030 should redirect the B cell response towards the remaining Bexsero antigens (NHBA-GNA-2091, NadA and PorA P1.4). It is however unclear as to why there are no detectable antibodies against NHBA-GNA-2091, NadA and PorA P1.4 in sera of Vax2 and Vax4 at titer 1:200 and no specific reactive Igs in the plasmablast populations of both vaccinees. This data however supports earlier conclusion from serum data, that fHbp-GNA1030 must be the most immunodominant Bexsero antigen.

It is also unclear as to why all Bexsero-reactive Igs reactive with structurally different antigens (insulin, LPS and dsDNA) failed to interact with any of the individual Bexsero antigens in the western blot analysis. One plausible hypothesis could be that all polyreactive Vax2, Vax3 and Vax4 Igs bind to non-continuous epitopes (proteins are linearized on reducing gel of western blot). It is however, highly unlikely that all polyreactive Igs across all three vaccinees interact with non-continuous epitopes. On the contrary, these Igs are polyreactive Igs aspecific to any of the Bexsero antigens, hence the lack of binding in western blot. All Vax2 and Vax4 Bexsero-reactive Igs belong in the polyreactive Ig population. The frequency of anti-NHBA-GNA2091 Igs (22/91) compared to anti-fHbp-GNA1030 Igs (2/91) and anti-NadA Igs (2/91) suggests a predominant anti-NHBA-GNA2091 response post second primary vaccination in the Vax3 anti-Bexsero response. The relatively low frequency of anti-fHbp-GNA1030 Igs raises questions about the strong anti-fHbp-GNA1030 Ig response observed in serum of Vax3. This observation buttresses earlier “epitope masking” hypothesis alluding to interference by pre-existing serum anti-fHbp-GNA1030 antibodies from the first primary vaccination, post second primary vaccination. The low frequency of the Vax3 anti-NadA monoclonal Igs and the lack of monoclonal Igs against PorA P1.4 mirrors the very weak detected serum anti-NadA and anti-PorA P1.4 Igs at titer 1:200. This overall observation supports earlier hypotheses of selective antigen targeting by the human immune system in the face of multiple antigens. 42.9% (39/91) of all expressed Vax3 anti-Bexsero Igs including members of cluster 4 are unreactive to Bexsero despite significant evidence of affinity maturation. Kuraoka et al, 2016 hypothesize that complex antigens are

susceptible to protein degradation, leading to a new B cell population potentially specific to novel non-native protein epitopes called "dark antigens" [29]. Wrammert et al, 2008 also in influenza vaccination where a substantial fraction of the antibody-producing B cells generated in response to whole influenza virus in humans were shown to have no detectable antigen-binding capacity suspected a myriad of factors including errors in RT-PCR, responses to denatured vaccine antigens, bystander activation of non-specific memory B cells or displacement of non-specific plasma cells from the bone marrow [66]. In conclusion, despite evidence of affinity maturation with highly biased selection patterns in the Ig populations of all three vaccinees, the Igs exhibit a wide range of specificities comprising of Bexsero-specific-reactive antibodies (Vax3 Only), polyreactive antibodies (Vax2, Vax3 and Vax4) and unreactive antibodies (Vax2, Vax3 and Vax4) and affinities also comprising of highly binding antibodies, moderately binding antibodies, weakly binding antibodies and unreactive antibodies (also observed in clonally expanded antibodies of Vax3 and Vax4).

Vax3 Bexsero-Specific Reactive Igs (Ig Gene & Reactivity Properties Analysis)

Selection is a crucial hallmark in shaping the B cell repertoire response to infections or vaccinations. Antigen-specific Bexsero-reactive antibodies were assessed for selection markers based on Ig gene family usage patterns, somatic hypermutation, isotype distribution and CDR3 length distribution. Cluster antibodies (cluster 1: 2 antibodies, cluster 2: 2 antibodies, cluster 3: 4 antibodies and cluster 5: 4 antibodies) disproportionately constitute 63.6% (14/22) of the predominant anti-NHBA-GNA2091 antibodies in the Vax3 Bexsero response, suggesting emphasis on clonally selected anti-NHBA antibodies in the B cell response to Bexsero. Antigen-specific Bexsero-reactive antibodies appear to originate from a mix of newly recruited mature naïve B cells with low SHM load and memory B cells with moderate SHM load from the first primary vaccination or polyreactive antibodies recruited into the anti-Bexsero response with moderate SHM load which may have accumulated new mutations during affinity maturation to become NHBA-specific. There also appears to be no unique selection pattern in the Ig isotypes between Bexsero-reactive Igs and the unreactive Igs, albeit all the Ig isotypes have been shown to activate one or more of the complement pathways at varying degrees of efficiency. The *IgVH* gene family usage pattern in the Bexsero-reactive Ig population indicated a significant differential selection from both the mature naïve population and the Vax3 unreactive Ig population whereas the *IgJH* gene family usage pattern only indicated a significant differential selection pattern from the mature naïve population. The usage of rarely used VH2, VH7 and JH1/2, although not unique to anti-Bexsero antibody response, suggests extensive usage of commonly used *IgVH* and *IgJH*

genes drove the B cell response towards rarely used *IgVH* and *IgJH* genes, supporting the immensely diverse nature of the Ig response to Bexsero. Within the anti-NHBA antibody population, high binding antibodies belong in the cluster antibodies whereas moderately binding and weakly binding antibodies are distributed between both cluster and non-cluster antibodies. Anti-NadA and anti-fHbp antibodies are mainly made up of moderately binding antibodies. The relatively skewed nature of B cell response to anti-NHBA antibodies suggests preferential immunoselectivity within the Bexsero antigen pool. NHBA may be the most immunodominant Bexsero antigen after fHbp, already suggested to have been masked by anti-fHbp antibodies from the first primary vaccination. Within the anti-NHBA antibody population, 14 of the 22 antibodies belong in four clusters suggesting a maximum of four different epitopes targeted by these antibodies whereas the remaining eight non-cluster antibodies may also target a maximum of 8 different epitopes that may or may not overlap with epitopes targeted by the cluster antibodies. The anti-NHBA antibody response therefore appears restricted, also suggesting epitope immunodominance. In conclusion, the immune response to Bexsero induces antibodies against the different Bexsero antigens albeit at varying degrees, with varying antibody affinities and specificities suggesting immunoselectivity and antigen/epitope immunodominance.

Within the NHBA-specific Igs, it appeared there is a dual-population split between weak binding anti-NHBA Igs and high binding anti-NHBA Igs. Both populations exhibit a mix of both lowly mutated and highly mutated Igs, signifying a mix of newly recruited mature naïve B cells that accumulated SHM from a few rounds of mutations and memory B cells from the first primary vaccination accumulating a second series of mutations. Both germ line un-mutated polyreactive mature naïve B cells and existing moderately or highly mutated polyreactive B cells may also be recruited into the B cell response to Bexsero. Polyreactive Igs may obtain specificity to an antigen by acquiring crucial antigen affinity-altering mutations during affinity maturation. Polyreactivity may also be acquired by antigen-specific Igs during affinity maturation. Evidence of polyreactivity is observed in N6PMPB345, which binds either weakly or moderately to insulin, LPS and dsDNA in addition to Bexsero. High binding cluster 3 Igs also exhibit varying degrees of polyreactivity albeit slightly, which appears pronounced in N6PMPB369 and declines in other members of cluster 3 with acquisition of crucial affinity-altering mutations in both heavy and light chain CDR3 regions. However to ascertain if these anti-NHBA Igs are of polyreactive origin or acquired polyreactivity during affinity maturation, the Igs could be re-expressed at the germ line nucleotide sequence level. Tiller et al, 2007 have shown that a third of all polyreactive Igs are

polyreactive at germ line level [67]. On the contrary, Dal Porto et al, 2002 have shown that very low affinity B cells form germinal centers, become memory B cells and participate in secondary immune responses when higher affinity competition is reduced [68]. The two populations of weakly binding and strongly binding Igs show no unique *IgVH* gene usage but usage of rarely used *IgVH2* in the anti-NHBA Ig population suggests exhaustive exploitation of *IgVH* gene resources in the B cell response to Bexsero. Both populations also exhibit diversity in isotype distribution, suggesting functional diversity. The anti-fHbp and anti-NadA antibodies are lowly mutated, suggesting engagement of new mature naïve B cells with fHbp and NadA. *IgVH7* usage in one of the two anti-fHbp antibodies confirms general exhaustion of commonly used *IgVH* Ig genes.

Binding of Bexsero-Specific Reactive Igs to *Neisseria Meningitidis* mc58 in vitro

The poor binding affinities of the anti-NHBA antibodies to endogenous NHBA on *Neisseria Meningitidis* mc58, despite highly binding to recombinant NHBA in ELISA was unexpected. NHBA comprises of approximately 450 residues [31,69]. The N-terminal region (residues 1 to ~ 230) is intrinsically unfolded (disordered) whereas C-terminal region (residues 246 – 428) is a single 8-stranded anti-parallel beta-barrel, both connected by an arginine-rich motif (residues 235-245) [69]. Novartis Bexsero clinical trials reports human serum anti-NHBA antibody bactericidal activity at titers ≥ 1:4 [42]. Domina et al, 2016 have reported epitope mapping of a monoclonal antibody directed against NHBA using next generation sequencing of antigen-specific libraries [70]. Partridge et al 2017 have also shown the role of antibodies to NHBA in protection from polyclonal serum anti-NHBA antibodies from individuals vaccinated with Bexsero [71]. Maritan et al, 2017 have described the crystal structures of human Fabs targeting the NHBA and also providing indirect evidence of the disordered nature of the two N-terminal regions of NHBA [72]. However to date, no work has been published on human monoclonal antibodies against NHBA showing their reactivity or affinity profiles. Regardless, it appears the poor binding affinity of the anti-NHBA antibodies is a consequence of the disordered nature of the NHBA structure. Intrinsically disordered proteins lack a stable three-dimensional structure, but exist as an ensemble of interconverting conformers in solution, making them difficult to crystallize [73]. Intrinsically disordered proteins often undergo transient binding-induced folding upon interaction with specific binding partners [74]. As a result of these attributes, the interactions of disordered proteins are often of relatively low affinity, despite maintaining high specificity and for that matter distinct from the properties of typical antibody-antigen interactions, which are selected for high affinity. It has therefore been suggested that, disordered antigens may be poor

immunogens with established observations that functionally important sites on protein antigens are typically highly flexible, or bounded by flexible loops [75–77]. Kwong et al, 2002 propose that this flexibility serves as a means of immune evasion, with ‘‘conformational masking’’ mediated by the entropic cost of inducing order in the otherwise flexible antigen [76]. On the contrary, however, intrinsically disordered antigens can also be immunodominant. It has been shown that in some instances, despite being immunodominant, they fail to contribute to immune protection and are suspected to function as a decoy, diverting the immune system from targets with greater protective potential [78]. Additionally, the disordered nature of NHBA presents an otherwise important possibility of difference in conformation between endogenously expressed NHBA and recombinant NHBA used in Bexsero. Morales et al, 2015, have shown this in their work on the structural basis for epitope masking and strain specificity of a conserved epitope in intrinsically disordered Merozoite surface protein 2 (MSP2) [79]. MSP2 has been shown to elicit strain-specific antibody response in humans. The antibodies typically target the conserved N - and C – terminal regions of MSP2, but these antibodies react poorly with the native antigen on the parasite surface [79]. Morales et al, 2015 demonstrate that the recognition of a conserved N-terminal epitope by mAb 6D8 is incompatible with the membrane-bound conformation of that region, suggesting a mechanism by which native MSP2 escapes antibody recognition [79]. Finally, Guy et al, 2015, using bioinformatics tools have also predicted that intrinsically disordered proteins contain relatively few MHCI and MHCII binding peptides owing to inherent differences in amino acid composition compared to structured domains [80]. This property has a negative impact on peptide presentation by MHC molecules, which play an important role in adaptive immunity. This myriad of factors all together may explain the weakly binding affinities of the anti-NHBA antibodies to endogenous NHBA, which were otherwise strongly binding to recombinant NHBA in ELISA. Overall, this observation points to a need for detailed analysis of antigens for their ordered/disordered properties during vaccine development. The anti-fHbp and anti-NadA antibodies were moderately binding antibodies to recombinant fHbp and NadA respectively in ELISA and for that matter not completely unusual to observe weak binding of these antibodies in vitro to their endogenously expressed forms, although fHbp is a stable ordered antigen whereas the structure of NadA is currently not crystallized.

Further characterization of the functional role of these antibodies was not pursued, although the isolated anti-NHBA antibodies may be relevant in structural studies of NHBA. Regardless, Partridge et al, 2017 have shown with serum antibodies purified from individuals vaccinated with Bexsero, that anti-NHBA antibodies can exhibit bactericidal properties,

particularly in concert with anti-fHbp antibodies [71]. However, some high NHBA-expressing strains are resistant to anti-NHBA antibodies [71].

Recruitment of Polyreactive Antibodies into the B Cell Response to Bexsero

Polyreactive antibodies have been shown to acquire reactivity to antigens involved in immune response mostly from precursors that acquired mutations through somatic hypermutation [67]. Memory B cells recruited into the B cell response to Bexsero from the first primary vaccination may also become polyreactive from SHM accumulation during affinity maturation.

SHM & Isotype: Analysis of VH, VK and VL genes SHM distribution in Vax3 polyreactive Ig population in comparison with the Bexsero-antigen-specific Vax3 Ig population appears to indicate a higher average number of accumulated mutations in both VH and VK genes in the polyreactive Igs. A similar observation was made when Vax2 and Vax3 polyreactive Igs are compared with their corresponding unreactive Igs. The polyreactive Igs in all three vaccinees are mostly made of highly mutated Igs. Assessment of Vax3 isotype distribution in the polyreactive Ig population in comparison with the Bexsero-antigen-specific reactive Ig population shows selection of IgG4 and IgA2, both absent in the Bexsero-specific reactive Ig population. On the contrary, when compared with the Vax3 unreactive Ig population, the Ig isotype distribution is very similar between the polyreactive Ig population and unreactive Ig population. The Vax2 polyreactive Ig population also appears similar in Ig isotype distribution in comparison with the Vax2 unreactive Ig population, albeit, there is a significant difference in the differential expression of IgA2 and IgG1 between both Ig populations. In general, there is no unique selection pattern in isotype distribution in the polyreactive Ig populations in relation to the complete Ig population. Regardless, the selection of isotypes, different from those of Bexsero-antigen specific reactive antibodies in the polyreactive Ig population adds to the overall functional diversity in the Ig population recruited in response to Bexsero, given the ability of different Ig isotypes to activate one or more of the complement pathways albeit at different efficiencies.

VH, JH & CDR3 Length: In comparison with the mature naïve Ig population, polyreactive Ig populations from Vax2, Vax3 and Vax4 also appear to indicate equilibrium shifts in frequency distribution of classically selected *IgVH* and *IgJH* genes in the mature naïve Ig population. These differential changes in Ig gene usage appeared pronounced in Vax2 Ig (VH3, VH4, VH5/7), Vax2 Ig (JH4, JH5, JH6), Vax3 Ig (JH (1 – 3), JH5) and Vax4 Ig (JH5, JH6). In comparison with their corresponding unreactive Ig population, the differential changes in the polyreactive Ig population also appear pronounced in Vax2 Ig (VH1, VH4,

VH5/7), Vax3 Ig (VH1, VH3, VH4, VH5-7), and Vax3 Ig (JH (1 – 3) and JH5). A third comparison between Vax3 polyreactive Igs and Bexsero-antigen-specific Igs indicated significant differential changes in Ig (VH1, VH2 and VH3) and Ig (JH (1 – 3), JH4, JH5 and JH6). CDR3 Length analysis between the polyreactive Ig population and unreactive Ig population in both Vax2 and Vax3 Ig population indicate significant differential changes between the two panels. The difference in CDR3 length between Vax3 polyreactive Ig population and Vax3 Bexsero-antigen-specific reactive Ig population is also significant. In conclusion, there is no common unique pattern in the Ig gene features selected by polyreactive Igs between the three vaccinees but polyreactive Igs of each vaccinee shows unique gene features distinct from mature naïve B cells and the unreactive B cell population which appears to indicate their purposive recruitment into the B cell response to Bexsero. Muellenbeck et al, 2013 have shown the recruitment of polyreactive antibodies into the human B cell response in malarial infections, which also have a functional role [81]. Balakrishnan et al, 2011 have also shown that, Dengue virus activates polyreactive, natural IgG B cells after primary and secondary infections [82]. Polyreactive antibodies have been shown to be a major driver in the broad antibacterial activity of the natural antibody repertoire [65]. Polyreactive antibodies lyse bacteria and enhance phagocytosis in the presence of complement [81]. They also bind to apoptotic cells and with complement, enhance the phagocytosis of these cells by macrophages [81]. Simulations by Chaudhury et al, 2014 showed that B cell affinity maturation enhanced antibody cross-reactivity in response to polyvalent antigens, suggesting an attempt at resource conservation [83]. Simulations by Childs et al, 2015 also showed that, availability of many epitopes or antigens reduces the affinity and relative breadth of the antibody repertoire [16], also suggesting resource conservation, which further supports the hypothesis that recruitment of polyreactive antibodies as seen in Vax2, Vax3 and Vax4 in response to Bexsero vaccinations may be significant to the response to multi-antigen complexes. In conclusion, the human B cell response to Bexsero recruits highly mutated polyreactive antibodies, which may be a resource-conservation phenomenon of the human immune system in response to multi-antigen complexes to achieve broad coverage with a relatively small population of antibodies.

Correlation between Ig gene features and Bexsero reactivity properties

Igs from all three vaccinees behave differently in reactivity to Bexsero antigens. Vax2 and Vax4 Igs are mostly polyreactive Igs that do not particularly show any common selection patterns in Ig gene usage, CDR3 length and SHM, albeit isotype selection of high binding Vax2 antibodies appear to focus on IgG1 and IgG3. Vax3 antibodies on the contrary

comprises of both polyreactive antibodies and Bexsero-specific reactive antibodies. High binding Vax3 antibodies are predominantly specific Bexsero-reactive antibodies and mostly *IgVH1, IgVH2* or *IgVH4* and also *IgJH4*. These antibodies are mostly of the IgG1 isotype with CDR3 length between 10 – 14 amino acids and SHM ≤9. The absence of a strong correlation between Ig gene features and Ig reactivity within Vax2, Vax2 Ig subpopulations (polyreactive antibodies and unreactive antibodies), Vax3 and Vax3 subpopulations (Bexsero-specific reactive antibodies, polyreactive antibodies and unreactive antibodies) appear to buttress the existing challenge in predicting Ig reactivity from Ig gene features. There appears to be no common usage pattern in Ig gene features of antibodies recruited into the B cell response to Bexsero across all vaccinees and also in relation to Ig reactivity properties. Regardless, B cells recruited into the response to Bexsero exhibit immensely expanded diversity in Ig gene populations selected, with a wide range of affinities and specificities to the various Bexsero antigens. These comprises of Bexsero-specific reactive antibodies (Vax3 only), Polyreactive antibodies (Vax2, Vax3 and Vax4) and unreactive antibodies (Vax2, Vax3 and Vax4). This attribute collectively offers potential advantages for broad protection as expected from multi-antigen complexes. Kuraoka et al, 2016 have shown in mice that complex antigens elicit diverse patterns of clonal selection in germinal centers showing pools of antigen-reactive and unreactive antibody populations despite commonality in Ig gene selection biasness, clonal expansion and V(D)J mutations [29].

Immunodominance and Epitope Masking in the Anti-Bexsero Antibody Response

Vax2 and Vax4 must have reacted poorly to specific Bexsero antigens post-second primary vaccination, recruiting instead polyreactive antibodies at low plasmablast frequencies of 0.46% and 0.43% respectively. The absence of Bexsero-specific antibodies in the plasmablast data strongly suggests that serum antibodies against fHbp-GNA1030 must have originated from the response to the first primary vaccination. It is also highly improbable to have missed the peak plasmablast response time. Bergström et al, 2017 have shown in mice that IgG administered in close temporal relationship to antigen, inhibits all pathways of B cell differentiation [84]. Serum IgG as well as extrafollicular B cells, non-GC B cells, GC B cells, long-lived plasma cells, and memory B cells are all suppressed [84]. Secondly, they provide strong evidence for the importance of epitope masking and suggest that the role of IgG-mediated antigen clearance is negligible [84]. Getahun et al, 2009 also immunized mice with sheep red blood cells (SRBC) conjugated to hapten 5-iodo-4-hydroxyl-3-nitrophenacetyl (NIP) at high or low density and investigated the ability of IgG anti-NIP to suppress the Ig response to NIP and SRBC [85]. They showed that only NIP-specific response was suppressed

when mice were immunized with SRBC-NIP_{low}, whereas both NIP- and SRBC-specific responses were suppressed when immunized with SRBC- NIP_{high} [85]. This result suggests that both AG clearance and epitope masking occurs during IgG-mediated suppression, but that under physiological conditions, epitope masking is the predominant mechanism [85]. The presence of a strong predominantly IgG anti-fHbp antibodies and potentially anti-NHBA, anti-NadA and anti-PorA P1.4 albeit detectable probably at higher titers may have completely abrogated the response to the second primary vaccination in Vax2 and Vax4, recruiting only polyreactive antibodies. Vax3 on the contrary reacted strongly to specific Bexsero antigens post-second primary vaccination, recruiting both antigen specific antibodies (NHBA-GNA2091, fHbp-GNA1030 and NadA) and polyreactive antibodies at a plasmablast frequency of 3.76 %. It is however unclear as to why Vax3 mounted a response against other Bexsero antigens with specific focus on NHBA but not Vax2 and Vax4. The difference may arise from several factors including the genetic diversity of a host's naïve B cell population, stochastic founding events in germinal centers, genetic predisposition mediated by helper T cells and chance mutation during affinity maturation, although it's unclear what the relative contributions of these factors are [16]. The low frequency (2/91) of anti-fHbp-GNA1030 antibodies strongly supports previous hypothesis on Vax2 and Vax4 that serum antibodies against fHbp-GNA1030 must have originated from the response to the first primary vaccination. The response to the second primary vaccination appear to target NHBA-GNA2091 with plasmablast antibody frequency of 22/91 and detectable serum antibodies against Bexsero. Serum anti-NadA antibodies are detectable very weakly at titer 1:200, mirroring the low frequency (2/91) of anti-NadA antibodies in the plasmablast population. Serum anti-PorA P1.4 antibodies are also detectable very weakly at titer 1:200 despite the absence of anti-PorA P1.4 antibodies in the plasmablast pool. This data appear to support the Novartis clinical trial report on Bexsero due to evidence of B cell response against three of the four main Bexsero antigens (NHBA-GNA2091, fHbo-GNA1030 and NadA) in plasmablast of Vax3 and serum antibodies against all four antigens in Vax3 sera albeit only weakly to NadA and PorA P1.4. In conclusion it appears the immune system selectively targets Bexsero antigens in an order of immunodominance. Immunodominant epitopes are select epitopes from complex antigens that initiate T-cell responses. Childs et al, 2015 have postulated through simulations, that cross-reactivity and immunodominance are two hallmarks of the B cell response to multi-antigen complexes [16]. fHbp-GNA1030 must be the most immunodominant Bexsero antigen with strong serum antibody response detectable in all

vaccinees (Vax2, Vax3 and Vax4). NHBA-GNA2091 may be the second most immunodominant antigen. However variability in the degrees of immunodominance can only be concretely established after sampling large numbers of vaccinees. Regardless, fHbp-GNA1030 is highly probable to be most immunodominant Bexsero antigen. Selective targeting of antigens is a property exhibited by the immune system upon exposure to multi-antigen complexes such as pathogen infections [86]. The differences in immunodominance may be primarily due to differences in accessibility to epitopes of the antigens. fHbp has been shown to comprise of an N-terminal domain of 8-beta strands forming a highly curved anti-parallel beta-sheet and a C-terminal that is a well-defined beta-barrel of 8 anti-parallel beta strands connected by a short linker [87–89]. NHBA on the contrary has a disordered intrinsically unfolded N-terminal region and a single 8-stranded anti-parallel beta-barrel C-terminal region [69,90]. The 3D structure of NadA is currently unknown, although modeling from the other Oca family members suggests a "head" domain and a long stalk region predicted to form a homotrimeric coiled-coil region [31]. The outer membrane vesicles (OMVs) comprises of vesicles rich in outer membrane proteins, lipids and periplasmic components [31]. Kim et al, 2014 used a reductionist cell-free antigen-processing system composed of defined components to characterize steps in antigen processing of pathogen-derived proteins or autoantigens [91]. Pathogen-derived antigens are sensitive to digestion by cathepsins. Sensitivity to digestion by cathepsins drives the capture of pathogen-derived epitopes by MHC II before processing and resistance to HLA-DM mediated dissociation preserves the longevity of the captured epitopes [91]. They show that immunodominance is established by higher relative abundance of the selected epitopes that survive cathepsin digestion either by binding to MHC class II and resisting HLA-DM mediated dissociation, or being chemically resistant to degradation by cathepsins [91]. On the contrary, non-dominant epitopes have a high propensity for degradation by cathepsins and exhibit poor resistance to HLA-DM mediated dissociation [91]. Although a myriad of other factors may influence the variability in immunodominance, it appears fHbp is the most structurally stable antigen with clearly defined fold and epitopes compared to the potential structural constraints to B cells in the structurally complicated antigens, hence fHbp dominance in the B cell response to Bexsero antigens in humans.

Conclusions and Future Directions

To conclude, the data pointed to an expansive diversity (isotype distribution, *IgVH* and *IgJH* gene usage, CDR3 length distribution and clonal selection) of Ig populations generated in response to Bexsero with unique Ig gene usage patterns in all three vaccinees. Secondly, Ig populations that have undergone affinity maturation with biased selection patterns that exhibit a wide range of specificities comprising of Bexsero-specific-reactive antibodies (Vax3 Only), polyreactive antibodies (Vax2, Vax3 and Vax4) and unreactive antibodies (Vax2, Vax3 and Vax4) and affinities comprising of highly binding antibodies, moderately binding antibodies and weakly binding antibodies are shown. No unique correlation between specific Ig gene features and Ig reactivity properties was observed, supporting existing difficulty in predicting Ig reactivity from Ig gene sequences, albeit Igs from all vaccinees collectively exhibit varied affinities within clusters, between clusters and amongst non-clusters to Bexsero, with potential advantages for broad protection. Ig gene features and antigen-reactivity properties of antibodies generated against NHBA (22 antibodies), fHbp (2 antibodies) and NadA (2 antibodies) are shown. These antibodies exhibited poor binding affinities when tested on endogenously expressed antigens on Neisseria menigitidis mc58, potentially due to disordered N-terminal of NHBA, hence ending their further characterization. However, these antibodies may be important for the structural characterization of NHBA. The data also pointed to enrichment of highly mutated polyreactive antibodies in the B cell response to Bexsero, which may be a hallmark of the response to multi-antigen complexes to achieve broad coverage with a relatively small pool of antibodies. Varying degrees of immunoselectivity to the different Bexsero antigens, suggesting antigen immunodominance, with implications on the quality of the B cell response to Bexsero was also observed. Finally, the data also pointed to evidence of "epitope masking", observed post second primary vaccination with Bexsero also with implications on the quality of the B cell response to Bexsero and raises important discussions on booster/secondary vaccinations. Regardless, from the data on serum bactericidal assay during clinical trials, Novartis concluded the need for two doses in adults at one-month interval to achieve broad antibody coverage against all four antigens. It appears upon exposure to multi-antigen complexes comprising of antigens with varied antigenicity, immunoselectivity/immunodominance ensures that, the B cell response focuses on select antigen(s) post the first primary vaccination whereas epitope masking and phagocytic clearance of antibody:antigen complexes refocuses the B cell response to remaining antigen(s) in subsequent vaccinations to ensure the generation of antibodies against all/most

antigens of a multi-antigen complex. The interplay between these two phenomena, may explain why it is often necessary to offer more than one primary vaccination in order to effectively elicit antibody response against each antigen of a multi-antigen complex. From this data, an alternative approach to effectively elicit antibody response against each antigen would be to sequentially introduce each antigen as a separate vaccine to circumvent the complexity in the human B cell response in the face of multi-antigen complexes. The single cell analysis of plasmablast and serum analysis post-Bexsero vaccination buttresses the Novartis vaccination dosage and time-interval requirement and suggests the immunological phenomena responsible for defining this requirement.

Consequently, this controllable system of 4-antigen multi-antigen complex lays the framework for exploring in detail, the behavior of B cells in response to multi-antigen complexes (pathogen infections and vaccinations) in humans and evinces the need for expansive understanding of the fine cellular details of the immune response to vaccine antigens during vaccine clinical trials. An important experiment would be to purify and determine the binding affinities and bactericidal behavior of serum antibodies against Bexsero antigens. Despite the weak binding affinities of monoclonal antibodies generated against Bexsero antigens, the bactericidal behavior of these antibodies individually and in tandem with antibodies from other antigens would be interesting to study. Epitope mapping of purified serum antibodies and monoclonal antibodies from plasmablast to determine immunodominant and immune-relevant epitopes of the antigens. Ultimately, access to the sera and plasmablast from the first primary vaccination would provide a more robust profile of the human B cell response to Bexsero. Finally, it would be interesting to repeat this experiment with other multi-antigen complexes to ascertain the overarching behavior of human B cells in the face of multi-antigen complexes.

A more comprehensive study on the B cell response to multiple antigens could be assessed by investigating germinal center B cells, follicular dendritic cells, and plasmablast for the distribution of the different peptides carried by the follicular dendritic cell population, the peptide:MHCII complexes on germinal center B cells and antigen reactivity profiles of plasmablasts post vaccination with multiple antigen vaccines in suitable mouse models. In comparison with the B cell response to sequential vaccination of the single antigen components of the multiple antigen complex, the role of "immunoselectivity/immunodominance" and "epitope masking / antigen clearance by phagocytes in antibody:antigen complexes" upon vaccination with multi-antigen complexes can be extensively ascertained.

Chapter 4: Materials & Methodology

4a: Materials

4a.1 Vaccine

Bexsero is a multicomponent vaccine designed by Novartis Vaccines and Diagnostics S.r.l against *Neisseria meningitidis* serogroup B. The main active substances are recombinant Neisseria Heparin Binding Antigen (NHBA) fused to GNA1030, recombinant Neisseria Adhesin A (NadA), recombinant factor H binding protein (fHbp) fused to GNA2091 and Outer membrane vesicles (OMV) from *Neisseria meningitidis* group B strain NZ98/254 measured as amount of total protein containing the PorA P1.4. Bexsero is recommended for active immunization of individuals from 2 months of age and older against invasive meningococcal disease caused by *Neisseria meningitidis* group B.

NadA: A surface exposed oligomeric protein belonging to the Oligomeric Coiled-coil Adhesin, involved in binding to epithelial cells[33].

NHBA-GNA1030 fusion protein: Fusion protein product of Neisseria Heparin Binding Antigen and GNA1030, an accessory protein that enhances the immunogenicity of NHBA[31].

fHbp-GNA2091 fusion protein: Fusion protein product of Neisseria Heparin Binding Antigen and GNA2091, an accessory protein that enhances the immunogenicity of fHbp[31].

OMV (NZ98-254): Outer membrane vesicle (OMV) of *Neisseria meningitidis* strain NZ98-254[31].

Table 2: Composition of Bexsero

Component	**Quantity per 0.5 mL dose**
NadA	50 ug
NHBA-GNA1030	50 ug
OMV (NZ 98/254)	25 ug
Aluminum Hydroxide	1.5 mg
Sodium Chloride	3.125 mg
Sucrose	10 mg
Histidine	0.776 mg
H_2O	Up to 0.5 mL

Table 3: Bexsero dosage and vaccination scheme

Age Group	Primary Vaccination	2nd Primary Vaccination	Booster Vaccination
Infants: 2 - 5 months	Three doses (first at 2 months old)	Not less than 1 month	1 dose between 12 to 15 months after primary series
Infants: 6 - 11 months	Two doses	Not less than 2 months	1 does in second year after primary series
Children: 12 - 23 months	Two doses	Not less than 2 months	1 dose at least 12-23 months after primary series
Children: 2 - 10 years	Two doses	Not less than 2 months	Not established
Adolescents (from 11 years) & Adults	Two doses	Not less than one month	Not established

4a.2 Volunteers

Three healthy individuals (Adults) working in a laboratory setting with regular contact to multiple strains of *Neisseria meningitidis* were vaccinated with Bexsero (4CmenB), primarily for protection against *Neisseria meningitidis* infection. All three vaccinees designated Vax2, Vax3 and Vax4, voluntarily consented to providing blood samples post Bexsero vaccination to address the questions elaborated in this project.

4a.30. Blood Sample Collection Materials

4a.3.1. Disposable Syringe (Thermo Fisher Scientific)

4a.3.2. EDTA Vacutainer (BD)

4a.40. Ficoll: Materials and Reagents

4a.41. 50 mL Falcon tubes (Thermo Fisher Scientific).

4a.42. RPMI (Sigma-Aldrich)

4a.43. Ficoll-Plus (GE Healthcare)

4a.44. Pasteur pipette (Thermo Fisher Scientific)

4a.45. FCS (local source in Berlin),

4a.46. Hemocytometer (Neubauer Kammer)

4a.47. Trypan blue for cell counting (Thermo Fisher Scientific)

4a.48. DMSO (Sigma-Aldrich)

4a.49. Cryotubes (Sigma-Aldrich)

4a.410. Gradient freezing box (Mr. Frosty Freezing Container) (Thermo Fisher Scientific)

4a.411. 15 ml Falcon (Thermo Fischer Scientific).

4a.412. 1.5 mL Eppendorf tubes (Eppendorf)

4a.50. Staining Antibodies: Materials and Reagents

4a.5.1. CD19-Pe-Cy7 (BD Biosciences)

4a.5.2. CD27-PE (BD Biosciences)

4a.5.3. CD38-FITC (BD Biosciences)

4a.5.4. IgG-Pacific blue (BD Biosciences)

4a.5.5. 7-AAD (Life Technologies)

4a.5.6. Staining solution: Cold PBS (without Ca^{2+} or Mg^{2+}) with 2% heat-inactivated fetal calf serum (FCS) and anti-human antibodies or antigen at the final concentration.

4a.5.7. PBS/FCS: Cold PBS (without Ca^{2+} or Mg^{2+}) with 2% heat-inactivated FCS, sterile; store at 4°C.

4a.5.8. 7-Aminoactinomycin D (7-AAD) solution: Prepare a 2 mg/ml solution; store protected from light at 4°C.

4a.60. FACS Analyses and Single B Cell Sorting: Materials and Reagents

4a.6.1. 384 well plate (FrameStar)

4a.6.2. Lysis solution: 0.5× PBS (without Ca^{2+} or Mg^{2+}, molecular biology grade), 10 mM dithiothreitol (DTT), 8 U RNAsin® (Promega, Mannheim, Germany). Keep on ice until transferred to a 96-well PCR plate.

4a.6.3. Adhesive aluminum foil (BioRad).

4a.6.4. LSR II (BD Biosciences)

4a.6.5. ARIA II flow cytometric cell sorter (BD Biosciences)

4a.70. RT-PCR and Gene Amplification (PCR): Materials and Reagents

4a.7.1. PCR cycler compatible with 384-well PCR plates.

4a.7.2. Nuclease-free PCR water.

4a.7.3. Random hexamer primer (pd(N)6; Roche Applied Science, Mannheim, Germany): prepare a 300 ng/ml solution; store in small aliquots at −20°C.

4a.7.4. Igepal CA-630, molecular biology grade: prepare a 10% solution; store in aliquots at −20°C.

4a.7.5. RNAsin® (40 U/ml; Promega).

4a.7.6. SuperScript® III Reverse Transcriptase (200 U/ml; Invitrogen, Karlsruhe, Germany) with 5× RT buffer.

4a.7.7. DTT, molecular biology grade: prepare a 100 mM solution; store in small aliquots at −20°C.

4a.7.8. dNTP solution, prepare a mix with 25 mM of each nucleotide; store in small aliquots at −20°C.

4a.7.9. HotStarTaq® (5 U/ml; Qiagen, Hilden, Germany) with 10× PCR buffer.

4a.7.10. 5' primer or primer mix: prepare a working dilution of 50 mM (50 pmol/ml) for each primer. For primer mixes, combine equal volumes of 50 mM working dilutions of each primer (see Appendix for primer list); store in small aliquots at −20°C.

4a.7.11. 3' primer: Prepare a working dilution of 50 mM for each primer. For primer mixes, combine equal volumes of 50 mM working dilutions of each primer (See Appendix for primer list); store in small aliquots at −20°C.

4a.7.12. Adhesive seal (PCR suitable)

4a.7.13. Agarose

4a.7.14. TAE buffer: 40 mM Tris–acetate, 1 mM ethylenediamine tetra- acetic acid (pH 8.0).

4a.7.15. 10 mg/ml Ethidium bromide solution: dilute 1:2,000 in TAE buffer.

4a.7.16. Loading dye for DNA gels: 60% w/v sucrose, 1 mM Cresol Red

4a.7.17. 100 bp DNA ladder.

4a.80. Expression Vector Cloning: Materials and Reagents

4a.8.1. PCR cycler compatible with 96-well PCR plates.

4a.8.2. 5' primer: Prepare a working dilution of 5 mM for each primer (See Appendix for primer list).

4a.8.3. 3' primer: Prepare a working dilution of 5 mM for each primer (See Appendix for primer list).

4a.8.4. Adhesive seal, PCR suitable.

4a.8.5. Nuclease-free PCR water.

4a.8.6. HotStarTaq® (5 U/ml; Qiagen) with 10× PCR buffer.

4a.8.7. dNTP solution, prepare a mix with 25 mM of each nucleotide; store in small aliquots at −20°C.

4a.8.8. PCR purification kit, e.g., Qiaquick® 96 PCR Purification Kit (Qiagen) or NucleoSpin® 96 Extract II (Macherey and Nagel).

4a.8.9. *Age*I-HF (20 U/ml; New England Biolabs (NEB), Frankfurt/ Main, Germany) with NEBuffer 4 (10×) and BSA (100×).

4a.8.10. *Sal*I-HF (20 U/ml; NEB) with NEBuffer 4 (10×).

4a.8.11. *Bsi*WI (10 U/ml; NEB) with NEBuffer 3 (10×).

4a.8.12. *Xho*I (20 U/ml; NEB) with NEBuffer 4 (10×) and BSA (100×).

4a.8.13. NEBuffer 1 (NEB).

4a.8.14. Linearized human *IgH* g1 expression vector with cloning sites for *Age*I and *Sal*I, and ampicillin resistance gene (50 ng/ml; see Note 10).

4a.8.15. Linearized human *Igλ* k1 expression vector with cloning sites for *Age*I and *Bsi*WI, and ampicillin resistance gene (50 ng/ml; see Note 10).

4a.8.16. Linearized human *Igλ* l2 expression vector with cloning sites for *Age*I and *Xho*I, and ampicillin resistance gene (50 ng/ml; see Note 10).

4a.8.17. T4 DNA ligase (400 U/ml) with 10× T4 DNA ligase reaction buffer.

4a.8.18. Chemical competent *Escherichia coli* (*E. coli*) DH10B cells (Invitrogen).

4a.8.19. LB medium: 10 g bacto-tryptone, 5 g bacto-yeast extract, 10 g NaCl, adjust to pH 7 with NaOH and fill to 1 l with distilled H_2O; store at room temperature.

4a.8.20. LB agar plates containing 100 mg/ml ampicillin.

4a.8.21. Taq DNA polymerase (5 U/ml) with 10× PCR buffer.

4a.8.22. 5' Ab sense (See Appendix for primer list): Prepare a working dilution of 50 mM; store in small aliquots at −20°C.

4a.8.23. 3' primer (3' IgG (internal), 3' Ck 494, 3'Cl; See Appendix for primer list): Prepare a working dilution of 50 mM; store in small aliquots at −20°C.

4a.8.24. Terrific Broth containing 75 mg/ml ampicillin; pre-warmed to room temperature.

4a.8.25. Round bottom tube with snap cap, 13 ml.

4a.8.26. Plasmid purification kit, e.g., QIAprep® Spin MiniPrep Kit (Qiagen) or NucleoSpin® Plasmid (Macherey-Nagel, Düren, Germany).

4a.9 Ig Gene Sequencing and Sequence Analysis: Materials and Reagents

4a.9.1. DNA material sequenced by Eurofins GmbH (see details on Eurofins website | see Appendix for sequence primers)

4a.9.2. Sequence analysis done with NCBI IgBLAST (https://www.ncbi.nlm.nih.gov/igblast/)

4a.10 Expression of Recombinant Monoclonal Igs: Materials and Reagents

4a.10.1. Human embryonic kidney (HEK) 293T cells (ATCC, No. CRL-11268).

4a.10.2. 150 mm Cell culture plates.

4a.10.3. D-MEM growth medium: Dulbecco's Modified Eagle's Medium (D-MEM; GibcoBRL, Darmstadt, Germany) supplemented with 10% heat-inactivated FCS (Invitrogen), 100 mg/ml streptomycin, 100 U/ml penicillin G, and 0.25 mg/ml amphotericin B (all GibcoBRL).

4a.10.4. D-MEM wash medium: D-MEM (GibcoBRL) supplemented with 100 mg/ml streptomycin, 100 U/ml penicillin G, and 0.25 mg/ml amphotericin B (all GibcoBRL).

4a.10.5. D-MEM nutridoma medium: D-MEM (GibcoBRL) supplemented with 1% Nutridoma-SP (Roche Applied Sciences, Mannheim, Germany), 100 mg/ml streptomycin, 100 U/ml penicillin G, and 0.25 mg/ml amphotericin B (all GibcoBRL).

4a.10.6. 150 mM NaCl, sterile.

4a.10.7. Vector DNA: Equal amounts (10–15 mg) of Igg1 expression vector DNA and *Igk/Igλ* expression vector DNA.

4a.10.8. Polyethylenimine, branched (PEI; Sigma): Prepare a PEI solution of 1 mg/ml in deionized water, filter sterilize. Store at 4°C for up to 6 months.

4a.10.9. Sodium azide (NaN_3) solution.

4a.10.10. Protein G Sepharose™ (GE Healthcare, Munich, Germany).

4a.10.11. PBS, sterile.

4a.10.12. Chromatography spin columns (Bio-Rad).

4a.10.13. 0.1 M glycine, pH 3.0

4a.10.14. 1 M Tris, pH 8.0, supplemented with 0.5% NaN_3.

4a.11 Enzyme-Linked Immunosorbent Assay (ELISA): Materials and Reagents

4a.11.1. Goat anti-human IgG (Dianova)

4a.11.2. Bexsero (whole vaccine)

4a.11.3. ELISA plates (Costar)

4a.11.4. 2% BSA in PBS-B

4a.11.5. PBS-B (1× PBS, 0.005% vol/vol Tween-20, and 0.01% wt/vol EDTA)

4a.11.6. dsDNA

4a.11.7. LPS

4a.11.8. Insulin

4a.11.9. Human IgG1k from plasma of myeloma patients (Sigma – Aldrich)

4a.11.10. Goat anti-human IgG-HRP antibody (Dianova)

4a.11.11. ABTS substrate (Roche)

4a.11.12. SoftMax software (Molecular Devices)

4a.11.13. PRISM (GraphPad Software)

4a.11.14. ED38 antibody (Highly polyreactive monoclonal antibody expressed in-house)

4a.11.15. JB40 antibody (Moderately polyreactive monoclonal antibody expressed in-house)

4a.11.16. mGO53 antibody (Specific monoclonal antibody expressed in-house)

4a.12 Antigen Desorption: Materials and Reagents

4a.12.1 SDS in extraction buffer (0.60 M citrate, 0.55 M phosphate, pH 8.5)

4a.12.2 Daigger vortex genie 2 (Daigger & Co. Inc.)

4a.13 Western Blotting: Materials and Reagents

4a.13.1 SDS gel (Mini-PROTEAN® TGX™ Precast Polyacrylamide Gels).

4a.13.2 PVDF membrane (Thermo Fisher Scientific).

4a.13.3 Goat anti-human IgG-HRP (Jackson).

4a.13.4 ECL reagent (Pierce™ ECL Western Blotting Substrate).

4a.13.5 Semi-Dry Blotter (Daiger Scientific)

4a.13.6 1X Phosphate Buffered Saline (PBS).

4a.13.7 1X SDS Sample Buffer**:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT and 0.01% w/v bromophenol blue.

4a.13.8 Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5).

4a.13.9 10X Tris Buffered Saline (TBS): E.g. 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).

4a.13.10 Nonfat Dry Milk

4a.13.11 Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk: E.g. for 150 ml blocking buffer = 15 ml 10X TBS + 135 ml water, + 7.5 g nonfat dry milk + 0.15 ml Tween-20 (100%).

4a.13.12 Wash Buffer**:** 1X TBS, 0.1% Tween-20 (TBS/T).

4a.13.13 Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; E.g. for 20 ml antibody dilution buffer = 2 ml 10X TBS + 18 ml water + 1.0 g nonfat dry milk + 20 µl Tween-20 (100%).

4a.13.15 Prestained Protein Marker, Broad Range (Premixed Format).

4a.13.16 Blotting Membrane: PVDF membrane

4a.14 *Neisseria meningitidis* binding assays: Materials and Reagents

a. *N. meningitidis* mc58 **(**Albrecht and Ghon) Murray

b. Paraformaldehyde (Sigma-Aldrich).

c. 1X PBS, sterile.

d. PBS plus 0.1 % Tween 20 (PBS-T)

e. Fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (1:50 dilution: Jackson ImmunoResearch Laboratories, Inc., Bath, United Kingdom)

f. FACS Calibur fluorescence-activated cell sorter (Becton Dickson)

4a.15. Statistical Analysis

4a.15.1 PRISM (GraphPad Software)

4b: Methodology

4b.1. Vaccination

The vaccination was executed according to the scheme recommended by Novartis comprising of two Bexsero doses for adults, administered at least one-month apart [28, 30]. The first and second primary vaccinations were offered to the vaccinees at a one-month interval. The vaccinees were vaccinated intramuscularly from a pre-filled syringe with an attached needle in the Bexsero package.

NB: Prof. Christoph Tang vaccinated and subsequently drew blood from the individuals seven days post vaccination in his lab at the Sir William Dunn School of Pathology at Oxford University.

4b.20 Ficoll

Seven days post Bexsero-vaccination, 50 mL blood samples were taken from each donor, using syringe, into EDTA vacutainers (BD Biosciences). The fresh blood samples were then run over a Ficoll-Paque (GE Healthcare) centrifugation gradient to separate peripheral blood mononuclear cells (PBMCs) from sera, erythrocytes and other blood components.

4b.2.1 Ficoll Isolation Protocol

The blood samples were transferred from 15mL EDTA vacutainers (BD Biosciences) into 50 mL Falcon tubes (Thermo Fischer Scientific). Under the biological safety cabinet, each 15 mL blood sample was diluted in a 1:1 v/v ratio with pre-warmed RPMI (Sigma-Aldrich) in a 50 mL Falcon tube. 18 mL of the diluted blood samples was then layered on top of 18 mL Ficoll-Plus in a 50 mL Falcon tube by pipetting slowly with 10 mL tip pipette on to the plastic rim. This cycle was repeated until the diluted blood samples had ran out. The samples were then centrifuged at 1500 rpm, 24^{o}C for 40 minutes with the slowest acceleration and without break.

4b.2.2 PBMC isolation

The interphase (the cloudy layer between the yellowish serum and the above Ficoll) were collected in new 50 mL falcon tubes using Pasteur pipette (Thermo Fischer Scientific) by gently moving the pipette up and down the Falcon tube without disturbing the Ficoll gradient. RPMI was then added to top the volume of each falcon to 50 mL and centrifuged at 1450 rpm, 4^{o}C for 10 minutes. The cell pellets after centrifuging were kept on ice, supernatant discarded and the washing step repeated 1X time with RPMI. The cells were then resuspended with cold FCS and counted in a Hemocytometer (Neubauer Kammer), using a mix of 40µl cell suspension + 40µl trypan blue. After cell counting, the concentration of the

cell suspension was adjusted to 6 x 10^6cells/ml. This was followed by adding 500 uL aliquots of the cell suspension to 500 uL of cold freezing medium (80% FCS, 20% DMSO tissue culture grade) to reach a final cell suspension of 3 x 10^6cells resuspended in 90% FCS 10% DMSO (Sigma-Aldrich). The cryotubes were quickly transferred into a gradient freezing box and stored at -80C freezer. After about 4 hours, the cryotubes were transferred from the gradient freezing box into a storage container in liquid nitrogen.

4b.2.3 Blood Serum Isolation The plasma-phase (top phase of the ficoll gradient) was carefully pipetted into a labeled 15 mL Falcon (Thermo Fischer Scientific). 1 mL aliquots of the sera were made into 1.5 mL eppendorf tubes (Eppendorf) and stored away at – 20^{o}C.

4b.30 Antibody staining protocol

Table 4: Composition of antibody staining mix

CD/Antigen	Expression marker	Kind of Ab &	Dilution	Vol/50 ul mix
CD19	All B cells	CD19 (Pe-Cy7)	1,5: 50	1,5 ul
CD38	Plasmablast/Memory B cells	CD10 (FITC)	1:25	2 ul
CD27	Memory Lymphocytes	CD 27 (PE)	1:25	2 ul
IgG	IgG+ Cells	IgG (V450/Pac Blue)	1:25	2 ul
		PBS-F (PBS with 2% FCS)		42.5 ul

The staining antibodies were mixed according to the above ratios and on ice. Frozen PBMCs were also transferred from liquid nitrogen on ice. Right on the verge of thawing, the cells were quickly transferred into 50 mL RPMI and centrifuged for 4 minutes at 4000xg. The cells were counted and distributed into 1.5 mL eppendorf tubes at 2 million cells per tube in 500 uL volumes. The eppendorf tubes were then centrifuged to pellet cells and discard RPMI. The PBMC pellets were incubated in the antibody mix (50 uL) for 30 minutes. This was followed by a washing step with 1mL of PBS/FCS, centrifuged at 4000xg, 4^{o}C and carefully discarded supernatant. The washing step was repeated and cell pellets resuspended in 50 mL of 7-AAD (1:200 dilution) for 30 minutes in the dark, to exclude dead cells. The washing step was repeated twice. The cells were then resuspended in 400 mL PBS/FCS, filtered through pre-wet 35 mm strainer cap into a round bottom tube and centrifuged for 1 minute at 8xg. The cells were kept on ice until loaded onto FACS machine or sorter.

4b.40 Flow Cytometry Analysis and Cell Sorting

Standard flow cytometry analyses of PBMC samples were conducted using LSR II (BD Biosciences). Plasmablast was sorted from the PBMCs using an ARIA II flow cytometric cell sorter (BD Biosciences). For **cell sorting:** the flow cytometer for the 384-well sorting was set up by sorting single droplets into a test 384-well plate. It was ensured that only a

single droplet was visible and located in the center of the bottom of each well. Plasmablasts from each vaccinee were sorted into the 384-well PCR plate prefilled with 2 uL of lysis buffer/RHP mix (see details below) per well. The plates were immediately sealed with Microseal F foil (BioRad) and put on dry ice before transferring to – 80 ^{o}C.

4b.4.1 Lysis buffer (with random hexamer primer mix)

Table 5: Composition of sorting /RHP buffer mix

Sort/RHP mix	**Reagent**	**Concentration**	**1x [uL]**	**420 x [uL]**
	Water	-	1.4813	622.13
	PBS	[10x]	0.0500	21.00
	DTT	[100 mM]	0.1000	42.00
	NP-40	[10%]	0.1375	57.75
	RHP	[300 ng/µl]	0.1375	57.75
	RNAsin	[40 U/µl]	0.0938	39.38
	Total		2.0000	840.00

NB: nuclease free water (Eppendorf), PBS (Promega), DTT (Promega), NP-40 (Sigma-Aldrich), RHP (GE Healthcare), RNAsin (Promega).

The Sort/RHP combination mix was prepared according to the constituent in the table above and distributed at 2 uL per well 384 well plates. The plates were sealed with aluminium plate seal (BioRad) and stored at -80^{o}C until sorting.

4b.50. Ig Gene Amplification (RT-PCR and PCR)

4b.5.1 cDNA Synthesis

Prior to working on the cDNA synthesis, the hoods, tables and pipettes were cleaned thoroughly with RNAway and DNAway detergents to get rid of both RNA and DNA from these surfaces. The RT-Mix (see table below) was prepared on ice and under the hood in strict RNAse-free conditions. The plates of single sorted cells were thawed on ice and span down in a centrifuge. The thawed plates were then incubated for 1 min at 68^{o}C and returned back onto ice. The microseal foil (BioRad) of the sort plate was carefully removed to add 2 uL of the RT-mix to each well. The plates were carefully mixed, sealed and run in a 384-well plate PCR machine to perform reverse transcription at 42 ^{o}C for 5 min, 25 ^{o}C for 10 min, 50 ^{o}C for 60 min, 94 ^{o}C for 5 min. The cDNA product of the reaction was stored at -20 ^{o}C until further use.

Table 6: Composition of reverse transcription PCR mix

RT mix	Reagent	Concentration	1x [uL]	420x [uL]
	Water	-	2.05	267.75
	First strand buffer	[5x]	3.00	336.00
	DTT	[100 mM]	1.00	126.00
	dNTPs	[25 mM each]	0.50	57.75
	RNAsin	[40 U/ul]	0.20	23.63
	SuperScript III	[200 U/µl]	0.25	28.88
	Total		7.00	840.00

NB: superscript (Invitrogen)

4b.5.2. Ig Gene Amplification

Human *IgH* and *IgK* or Igλ gene transcripts from the same cell are amplified separately by nested PCRs (primary and secondary PCRs). All PCR steps are conducted on ice while wearing gloves. The primary PCR mix was prepared according to the table (Primary PCR) below. 9.0 uL of primary PCR mix was transferred per well into 384-well plate. 1.0 uL of cDNA from the cDNA plate was transferred into the first PCR plate with master mix. The content of the plate was mixed carefully and sealed with PCR film. The plate was then run at 94°C for 15 min; 50 cycles at 94°C for 30 s, 58°C (*IgH* and *Igk*) or 60°C (Igλ) for 30 s, 72°C for 55 s; 72°C for 10 min. The secondary PCR mix was prepared according to the table (Secondary PCR) below. 9.0 uL of primary PCR mix was transferred per well into 384-well plate. 1.0 uL of cDNA from the cDNA plate was transferred into the first PCR plate with master mix. The plate was then run at 94°C for 15 min; 50 cycles at 94°C for 30 s, 58°C (*IgH* and *Igk*) or 60°C (Igλ) for 30 s, 72°C for 45 s; 72°C for 10 min. 2 uL of second PCR product mixed with 3 uL of loading dye was transferred into a 2% analytical agarose gel in TAE buffer containing ethidium bromide and run for 25 min at 120 V. The DNA bands were visualized under ultraviolet (UV) light with expected Ig gene sizes (450 bp for *IgH*, 510 bp for *Igk*, and 405 bp for *Igλ*).

Table 7: Composition of primary PCR mix

Primary PCR	Reagent	Concentration	1x [uL]	405x [uL]
	Water	-	7.7900	3154.95
	Buffer	[10x]	1.0000	405.00
	Primer A	[50 µM]	0.0325	13.16
	Primer B	[50 µM]	0.0325	13.16
	dNTPs	[25 mM each]	0.1000	40.50
	HotStar Taq	[5 U/µl]	0.0450	18.23
	Sub-total		9.00	3645.00
	Template		1.00	
	Total		10	

Table 8: Composition of secondary PCR mix

Secondary PCR	Reagent	Concentration	1x [uL]	405x [uL]
	Water	-	7.7900	3154.95
	Buffer	[10x]	1.0000	405.00
	Primer A	[50 μM]	0.0325	13.16
	Primer B	[50 μM]	0.0325	13.16
	dNTPs	[25 mM each]	0.1000	40.50
	HotStar Taq	[5 U/μl]	0.0450	18.23
	Sub-total		9.00	3645.00
	Template		1.00	
	Total		10	

NB: see PCR primer lists in materials and methods section. 10X Buffer (NEB), dNTPs (Invitrogen) and HotStar Taq (Qiagen)

4b.5.3 Analysis of PCR products, Sequencing and Sequence Analysis

The 384-well PCR plates were assessed for PCR success. The PCR data was compiled and matched for heavy chains and light (kappa and lambda) chains. Wells with matching heavy and light (kappa and/or lambda) chains were sent for Sanger sequencing. Sequencing prep: 2 uL of secondary PCR product + 13 uL of ddH2O = 15 uL (volume required by Eurofins) and 2 uL of corresponding secondary PCR primer(s) (see primer list below) at 10 uM. Ig gene sequences were analyzed using the Igblast (www.ncbi.nlm.nih.gov/igblast/) database to identify variable gene segments (heavy chain: V_H, kappa chain: V_K, and lambda chain: V_L), diversity gene segments (Heavy chain: D_H) and joining gene segments (heavy chain: J_H, kappa chain: J_K, and lambda chain: J_L). The somatic hyper-mutation (SHM) count of each Ig heavy and light chain and the complimentary determining region 3 (CDR3) amino acid sequence and length, of each Ig heavy chain were also collated. Using the ensembl (www.ensembl.org) database, the isotype of each heavy and light chain antibody sequence was annotated.

4b.6 Expression vector cloning

Successfully sequenced secondary PCR products with matching heavy and light (kappa and/or lambda) chain Ig genes were further processed for cloning. These secondary PCR products were used in "Ig Gene specific PCR" with Ig primers tagged with 5' and 3' restriction sites (*Age*I and *Sal*I for *Igh*, *Age*I and *Bsi*WI for *Igk*, and *Age*I and *Xho*I for *Igλ*) for cloning directly into expression vectors [92].

4b.6.1 Ig Gene-Specific PCR

A master PCR mix was prepared with 29.2 uL water, 4 uL of PCR buffer, 0.4 uL of dNTP solution and 0.2 uL of HotStar® Taq per well. The PCR mix was transferred into 96-well PCR plate at 33.8 uL per well. 2 uL of the 5' and 3' of respective gene-specific primers were transferred into the corresponding wells of the 96-well PCR plate. 4.2 uL of the respective first PCR product was added to the corresponding well of the 96-well PCR plate, mixed carefully and sealed. The PCR plate was run at 94°C for 15 min; 50 cycles at 94°C for 30 s, 58°C (*IgH* and *Igk*) or 60°C (Igλ) for 30 s, 72°C for 45 s; 72°C for 10 min. To verify PCR success, 4 uL of the PCR product was mixed with 3 uL of loading dye onto a 2% analytical agarose gel in TAE buffer containing ethidium bromide and run for 25 min at 120 V. The bands were then visualized under ultra-violet (UV) light with expected Ig gene sizes (450 bp for *IgH*, 510 bp for *Igk*, and 405 bp for *Igλ*).

4b.6.2 Ig Directional Cloning into Expression Vectors

The specific PCR products were purified using Qiaquick PCR purification kit (Qiagen) and QIAvec 96 and elute in 60 uL of water. 3.5 uL of NEBuffer 4 was added to 31.5 uL of purified PCR products of *IgH* and Igλ chain V genes whereas 3.5 uL NEBuffer 1 was added to the purified PCR product of *Igk*. Enzyme mixes for *IgH*, *Igk* and *Igλ* were prepared according to the table below. Enzyme mix one was added to the respect Ig chain PCR mix with NEBuffer, mixed carefully and sealed with PCR film. The mix was then incubated for 2hrs at 37^{O}C. For *Igk* V gene PCR products, a second enzyme mix (enzyme mix 2: see table) was prepared because the second enzyme (*BSi*WI) performs optimally at 55^{o}C. 2 uL of AgeI-digested *Igk* V gene PCR products was added to the mix, mixed carefully and sealed with PCR film. The plate was then incubated for 2hrs at 55^{o}C.

Table 9: Composition of *IgVH, IgVk and IgVλ* restriction digestion mixes.

	***IgH* V Gene PCR Products**	***Igk* V Gene PCR Products**	***Igλ* V Gene PCR Products**
Enzyme Mix 1	4.0 ml water	4.05 ml water	4.0 ml water
	0.5 ml NEBuffer 4	0.5 ml NEBuffer 1	0.5 ml NEBuffer 4
	0.4 ml BSA	0.4 ml BSA	0.4 ml BSA
	0.05 ml *Age*I	0.05 ml *Age*I	0.05 ml *Age*I

	0.05 ml *Sal*I		0.05 ml *Xho*I
Enzyme Mix 2		1.7 ml water	
		0.2 ml NEBuffer 1	
		0.1 ml *Bsi*WI	

The digested PCR products were purified using the PCR purification kit (Qiagen), and eluted in 60 uL of water. 8 uL of digested and purified gene-specific PCR product was ligated into 0.5 uL expression vector using 1 uL T4 DNA ligase (Invitrogen) reaction buffer and 0.5 uL T4 DNA ligase. The ligation mix was incubated for 2hrs at room temperature or overnight at 16^{o}C. 3 uL of ligation product was added to 5 uL of competent DH10B bacteria (Clontech) cells thawed on ice, mixed carefully and incubated at room temperature for 30 minutes. The bacteria/ligation mix was heat shocked for 45 seconds at 42 ^{o}C and returned on ice. 200 uL of LB medium was added to the bacteria/ligation mix and incubated at 37 ^{o}C for 30 minutes under moderate shaking. The bacteria were then plated on ampicillin containing LB agar (Sigma-Aldrich) plate. The plates were incubated upside down over night at 37 ^{o}C.

The insert check PCR was performed from a master mix of 19.1 ml water, 2.5 ml PCR buffer, 2.5 ml dNTP solution, 0.2 ml 5' Ab sense primer, 0.2 ml 3' primer, and 0.5 ml Taq DNA polymerase per well. 25 uL of the master mix was transferred to each well on a 96-well plate, placed on ice and inoculated with bacterial colonies. The colonies used for insert check PCR were labeled in order to screen bacterial colonies for the presence of appropriately sized inserts. The insert check PCR was run at 94°C for 15 min; 27 cycles at 94°C for 30 s, 58°C for 30 s, 72°C for 60 s; 72°C for 10 min. To verify PCR success, PCR products were run on a 2% agarose gel to verify amplification (expected PCR product sizes are 650 bp for Igg1 vector, 700 bp for *Igk* vector, and 590 bp for *Igλ* vector). The insert check PCR products with the expected sizes were sequenced using Ab sense as sequencing primer (see primer sequence below). Colonies with 100% sequence identity to the secondary PCR products were inoculated in 4 mL Terrific Broth (Difco Laboratories) containing 75 ug/mL ampicillin (Sigma) in round bottom tube and incubated for 16hrs at 37 ^{o}C, 200 rpm. The resulting plasmid DNA were then isolated from 2 mL bacteria culture using QIAprep Spin columns (Qiagen) and eluted in 75 uL elution buffer. The plasmid concentrations were determined at A_{260} and A_{260}/A_{280} ratios with a NanoDrop.

4b.7 Expression of Recombinant Monoclonal Antibodies

Recombinant monoclonal antibody expression was done using PEI-mediated transfection.

4b.7.1 Recombinant Antibody Production

Human embryonic kidney (HEK) 293 (ATCC, No. CRL-1573) or 293T (ATCC, No. CRL-11268) cells was grown in 150 mm plates (Falcon, Becton Dickinson) in 25 mL Dulbecco's Modified Eagle's Medium (DMEM) growth medium (supplemented with 10% heat-inactivated ultralow IgG fetal calf serum (FCS)(Invitrogen), 1 mM sodium pyruvate (GibcoBRL), 100 ug/mL streptomycin, 100 U/mL penicillin G and 0.25 ug amphotericin (all from GibcoBRL) under standard conditions (37 ^{o}C, 5% CO_2), ensuring that the cells were evenly spread across the plate. At 80% confluency, the medium was removed completely and the cells washed with 10 mL DMEM wash medium. The wash medium was completely removed and 25 mL DMEM supplemented with 1% Nutridoma-SP (Roche) was added to the cells on in the plate. The plate was then returned into the incubator. During the incubation period, a mix of NaCl, IgG1 vector and corresponding *Igk*/ *Igλ* vector obtained from Ig plasmid purification (ratio 150 mM NaCl: total vector DNA [ul/ug] = 50) was prepared. The mix was agitated on a vortexer, after which PEI was added (ratio PEI: total vector DNA [ug/ug] = 3) and immediately agitated again on the vortexer for 30 s. The transfection mix was then incubated for 10 minutes at room temperature. The PEI mixture was gently added drop-wise to each plate, aiming for even distribution of the mixture. The cells were cultivated for 4 days under standard conditions. To harvest, the supernatant of the culture was collected and centrifuged at 800xg for 10 min and transferred to a new reaction tube. Sodium azide was added to the harvested supernatant at a final concentration of 0.05% except for antibodies specifically expressed for functional assays. The samples were then stored at 4 ^{o}C.

4b.7.2 Recombinant Antibody Purification

To purify recombinant antibodies, 25 mL of protein G beads (GE Healthcare) per 25 mL of purified antibody, was equilibrated with PBS. The beads were then centrifuged for 10 minutes at 800xg, 4 ^{o}C. The beads:supernatant mix was cultured overnight at 4 ^{o}C under rotation. The next morning, chromatography spin columns were equilibrated with PBS. The protein G beads/supernatant culture was centrifuged for 10 minutes at 800xg, 4 ^{o}C. The supernatant was carefully removed and the beads resuspended in residual PBS in spin column. The columns were washed twice with 1 mL PBS. The recombinant antibodies were then eluted in three fractions (200 mL each) with glycine and collected in tubes each containing 20 uL Tris/sodium azide. (For functional assays, exclude sodium azide). The protein concentrations were measured using spectrophotometer and/or ELISA.

4b.8. Enzyme – Linked Immunosorbent Assay (ELISA)

Antibodies from the expression system are either used in the supernatant or purified into glycine/Tris buffer. ELISAs were performed as previously described in Tiller et al., 2008. Antigens were coated overnight at 4°C whereas goat anti-human IgG (Dianova) for concentration ELISA coated overnight at room temperature. For Bexsero (whole vaccine) and fHbp antigen ELISA's, ELISA plates (Costar) were coated at a concentration of 5ng/well of antigen. Plates were then blocked with 2% BSA in PBS-B (1× PBS, 0.005% vol/vol Tween-20, and 0.01% wt/vol EDTA) for 1hr followed by incubation with recombinant monoclonal antibodies for 2hrs at room temperature. Polyreactivity (cross-reactivity) ELISAs were performed by coating ELISA plates (Costar) with 10 ug/mL dsDNA or LPS or 5ug/ml insulin, 50 uL per well overnight at room temperature. Plates were then blocked with PBS-B for 1hr. Concentration ELISAs of recombinant monoclonal antibodies (purified or in supernatant) were performed by coating ELISA plates (Costar) with [2 ug/mL: 50 uL] goat anti-human IgG (Dianova) overnight at room temperature. Plates were then blocked with PBS-B for 1hr. Human IgG1k from plasma of myeloma patients (Sigma – Aldrich) was used as standard [dilution series] to determine concentrations of antibodies of interest. In all three ELISA types, bound recombinant monoclonal antibodies were detected using anti-human IgG-HRP antibody (Dianova). Anti-human IgG-HRP (Jackson) antibodies were detected using one-step ABTS substrate (Roche). Optical densities (OD) were measured at 405 nm and antibody concentrations were determined using softMax software (Molecular Devices)

Analysis of ELISA data including graphs and Area-under-curve (AUC) were mostly done using PRISM (GraphPad Software). Polyreactive antibodies are monoclonal antibodies that were determined to bind to at least two out of three of the structurally different antigens: dsDNA, insulin and LPS. Using high binding ED38 antibody, low binding JB40 and non-binding mGO53 antibody to insulin, LPS and dsDNA, the thresholds of polyreactivity were set[44-46].

4b.9. Antigen desorption protocol

Bexsero antigens were extracted from whole Bexsero in the presence of 30 mM sodium dodecyl sulfate in extraction buffer (0.60 M citrate, 0.55 M phosphate, pH 8.5) using standard antigen extraction protocol. The vaccine was briefly vortexed for 1.0 minute at 5.5 rpm on a Daigger vortex genie 2 (Daigger & Co. Inc.). 0.3 mL of Bexsero was then immediately transferred to an eppendorf microcentrifuge tube and 0.6 mL of extraction buffer (0.60 M sodium citrate dehydrate/0.55 M sodium phosphate dibasic with SDS, pH 8.5) was added. The tube was mixed by inversion 10 times and incubated for 2.5 hours at 60°C with gently

mixing every 20 minutes during the incubation. The tube was then centrifuged at 425 g for 2 minutes at room temperature. The supernatant was then transferred to a new microcentrifuge tube and used for analysis by SDS-PAGE and western blotting with remaining volume aliquoted in 5ul volumes and stored at -20^{o}C.

4b.10. Serum and Monoclonal Antibody screen on SDS gel (western blot)

3 ug/well of desorbed Bexsero in 20 uL volume of sample buffer was heated to 95 – 10 ^{0}C for five minutes, cooled on ice and microcentrifuged for 5 minutes. The sample was then loaded on an SDS gel (Mini-PROTEAN® TGX™ Precast Polyacrylamide Gels). The numbers of lanes run corresponded with the number monoclonal antibodies or sera to be tested. Each run included a lane for pre-stained protein marker to verify the quality of the electro-transfer. The content of the gel was electro - transferred onto PVDF membrane (Thermo Fisher Scientific). The PVDF membrane was blocked in blocking buffer overnight. The blocked membrane was washed three times in TBS/T and cut in into stripes (each stripe represents one lane of SDS gel run). (a) For monoclonal antibodies, 10 ug/mL of each antibody was tested against a stripe of the blocked membrane by incubating the membrane stripe for 4hrs in the antibody, diluted in antibody dilution buffer. (b) For serum antibodies, sera were diluted at 1:200 in antibody dilution buffer. Each stripe of blocked membrane was incubated in diluted serum for four hours. Either option was followed washing three times in TBS/T and 2hrs incubation in mouse anti-human IgG-HRP (Jackson) secondary antibody diluted in antibody dilution buffer at 1:1000. The membrane stripes were then washed three times in TBS/T and developed using ECL reagent (Pierce™ ECL Western Blotting Substrate).

4b.11. *N. meningitidis* binding assays

N. meningitidis mc58 for flow cytometry were grown overnight, collected, washed and then adjusted to an OD at 600 nm (OD_{600}) of 6 in PBS. The cells were fixed in 3% paraformaldehyde for 15 minutes at room temperature, and then washed three times with PBS. Subsequently, 10^7 CFU were incubated with dilutions of control or antibody of interest in a total volume of 100 uL for 30 min at 37 ^{0}C. The cells were then washed twice in 100 uL of PBS plus 0.1 % Tween 20 (PBS-T) and resuspended in PBS-T containing a fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (1:50 dilution: Jackson ImmunoResearch Laboratories, Inc., Bath, United Kingdom) and incubated for 30 minutes on ice. After a final wash, fluorescence was measured using FACS Calibur fluorescence-activated cell sorter (Becton Dickson), recording at least 10^4 events and the data compared with unstained cells (control sample). The result was analyzed in Flowjo and presented as the percentage of gated cells; the gate was set at around 5% of control cells incubated in PBST.

NB: Prof. Christoph Tang carried out this experiment in his lab at the Sir William Dunn School of Pathology at Oxford University.

4b.12. Statistical Analysis

P-values were calculated using Fisher's Exact Test, Chi-square test or non-paired two-tailed Student's t test.

4b.13. Study Approval

This study was conducted according to the Declaration of Helsinki principles. The team of Prof. Christoph Tang handled the ethical approval for this study in the UK. All participants recruited in this study clearly understood and consented to their participation.

Chapter 5: Bibliography

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Appendix

Primer Lists

Table 10: Ig Primary PCR Primers[50]

***IgH* Primary PCR**	
Primer	**Sequence**
Forward (Complete Mix)	
5'L-VH1	ACAGGTGCCCACTCCCAGGTGCAG
5'L-VH3	AAGGTGTCCAGTGTGARGTGCAG
5'L-VH4/6	CCCAGATGGGTCCTGTCCCAGGTGCAG
5'L-VH5	CAAGGAGTCTGTTCCGAGGTGCAG
VH3 Leader-A	TAAAAGGTGTCCAGTGT
IGHV1,7-X1	ATGGACTGGACCTGGAG
IGHV1-X1-041	TCCTCTTTGTGGTGGCAGCAGC
IGHV2-X1-036	TCCACGCTCCTGCTRCTGAC
RM-IGHV4-X1	ATGAAACACCTGTGGTTCTTCC
Reverse	
hCh-mu-outer	GGAAGGAAGTCCTGTGCGAGGC
3' CgCH1	GGAAGGTGTGCACGCCGCTGGTC
hCh-ep-outer	AAGGTCATAGTTGTCCCGTTGAGG
hCh-al-outer	TGGGAAGTTTCTGGCGGTCACG

***Igk* Primary PCR Primers**	
Primer	**Sequence**
Forward (Complete Mix)	
5' L-Vk1/2	ATGAGGSTCCCYGCTCAGCTGCTGG
5' L-Vk3	CTCTTCCTCCTGCTACTCTGGCTCCCAG
5' L-Vk4	ATTTCTCTGTTGCTCTGGATCTCTG
Reverse	GTCCGCTTTCGCTCCAGGTCACACT
3' Ck543	GTTTCTCGTAGTCTGCTTTGCTCA

***Igλ* Primary PCR**	
Primer	**Sequence**
Forward (Complete Mix)	
5' L-Vl1	GGTCCTGGGCCCAGTCTGTGCTG
5' L-Vl2	GGTCCTGGGCCCAGTCTGCCCTG
5' L-Vl3	GCTCTGTGACCTCCTATGAGCTG
5' L-Vl4/5	GGTCTCTCTCSCAGCYTGTGCTG
5' L-Vl6	GTTCTTGGGCCAATTTTATGCTG
5' L-Vl7	GGTCCAATTCYCAGGCTGTGGTG
5' L-Vl8	GAGTGGATTCTCAGACTGTGGTG
Reverse	
hCl-057	CACCAGTGTGGCCTTGTTGGCTTG

Table 11: Ig Secondary PCR Primers [50]

***IgH* Secondary PCR Primers**	
Primer	**Sequence**
Forward	
RMX2-A	AGGTGCAGCTGCTGGAGTCKGG
Reverse	
3' CmCH1	GGGAATTCTCACAGGAGACGA
hCh-al-inner	GTCCGCTTTCGCTCCAGGTCACACT
IgG-internal	GTTCGGGGAAGTAGTCCTTGAC
hCh-ep-inner	CCAGGCAGCCCAGAGTCACGG
Igκ Secondary PCR	
Primer	**Sequence**
Forward	
5' Pan-Vk	ATGACCCAGWCTCCABYCWCCCTG
Reverse	
3' Ck494	GTGCTGTCCTTGCTGTCCTGCT
***Igλ* Secondary PCR**	
Primer	**Sequence**
Forward	
RM-IGλV-A	CAGYCTGYSCTGACTCA
RM-IGλV-B	TCCTATGAGCTGACWCAG
Reverse	
hCλ-040	TCAGAGGAGGGYGGGAACAGAGTG

Table 12: Ig Specific PCR Primers [46]

***IgH* Specific PCR for Cloning**	
Primer	**Sequence**
Forward	
5′ AgeI VH1	CTGCA**ACCGGT**GTACATTCCCAGGTGCAGCTGGTGCAG
5′ AgeI VH1/5	CTGCA**ACCGGT**GTACATTCCGAGGTGCAGCTGGTGCAG
5′ AgeI VH3	CTGCA**ACCGGT**GTACATTCTGAGGTGCAGCTGGTGGAG
5′ AgeI VH3-23	CTGCA**ACCGGT**GTACATTCTGAGGTGCAGCTGTTGGAG
5′ AgeI VH4	CTGCA**ACCGGT**GTACATTCCCAGGTGCAGCTGCAGGAG
5′ AgeI VH 4-34	CTGCA**ACCGGT**GTACATTCCCAGGTGCAGCTACAGCAGTG
5′ AgeI VH 1-18	CTGCA**ACCGGT**GTACATTCCCAGGTTCAGCTGGTGCAG
5′ AgeI VH 1-24	CTGCA**ACCGGT**GTACATTCCCAGGTCCAGCTGGTACAG
5′ AgeI VH3-33	CTGCA**ACCGGT**GTACATTCTCAGGTGCAGCTGGTGGAG
5′ AgeI VH 3-9	CTGCA**ACCGGT**GTACATTCTGAAGTGCAGCTGGTGGAG
5′ AgeI VH4-39	CTGCA**ACCGGT**GTACATTCTGAAGTGCAGCTGGTGGAG
5′ AgeI VH4-39	CTGCA**ACCGGT**GTACATTCCCAGCTGCAGCTGCAGGAG
5′ AgeI VH 6-1	CTGCA**ACCGGT**GTACATTCCCAGGTACAGCTGCAGCAG
Reverse	
3′ SalI JH 1/2/4/5	TGCGAA**GTCGAC**GCTGAGGAGACGGTGACCAG
3′ SalI JH 3	TGCGAA**GTCGAC**GCTGAAGAGACGGTGACCATTG
3′ SalI JH 6	TGCGAA**GTCGAC**GCTGAGGAGACGGTGACCGTG
Igκ Specific PCR for Cloning	
Primer	**Sequence**
Forward	

5′ AgeI Vκ 1-5	CTGCA**ACCGGT**GTACATTCTGACATCCAGATGACCCAGTC
5′ AgeI Vκ 1-9	TTGTGCTGCA**ACCGGT**GTACATTCAGACATCCAGTTGACCCA GTCT
5′ AgeI Vκ 1D-43	CTGCA**ACCGGT**GTACATTGTGCCATCCGGATGACCCAGTC
5′ AgeI Vκ 2-24	CTGCA**ACCGGT**GTACATGGGGATATTGTGATGACCCAGAC
5′ AgeI Vκ 2-28	CTGCA**ACCGGT**GTACATGGGGATATTGTGATGACTCAGTC
5′ AgeI Vκ 2-30	CTGCA**ACCGGT**GTACATGGGGATGTTGTGATGACTCAGTC
5′ Age Vκ 3-11	TTGTGCTGCA**ACCGGT**GTACATTCAGAAATTGTGTTGACACA GTC
5′ Age Vκ 3-15	CTGCA**ACCGGT**GTACATTCAGAAATAGTGATGACGCAGTC
5′ Age Vκ 3-20	TTGTGCTGCA**ACCGGT**GTACATTCAGAAATTGTGTTGACGCA GTCT
5′ Age Vκ 4-1	CTGCA**ACCGGT**GTACATTCGGACATCGTGATGACCCAGTC
Reverse	**Sequence**
3′ BsiWI Jκ 1/4	GCCAC**CGTACG**TTTGATYTCCACCTTGGTC
3′ BsiWI Jκ 2	GCCAC**CGTACG**TTTGATCTCCAGCTTGGTC
3′ BsiWI Jκ 3	GCCAC**CGTACG**TTTGATATCCACTTTGGTC
3′ BsiWI Jκ 5	GCCAC**CGTACG**TTTAATCTCCAGTCGTGTC

***Igλ* Specific PCR for Cloning**	
Primer	**Sequence**
Forward	
5′ AgeI Vλ 1	CTGCT**ACCGGT**TCCTGGGCCCAGTCTGTGCTGACKCAG
5′ AgeI Vλ 2	CTGCT**ACCGGT**TCCTGGGCCCAGTCTGCCCTGACTCAG
5′ AgeI Vλ 3	CTGCT**ACCGGT**TCTGTGACCTCCTATGAGCTGACWCAG
5′ AgeI Vλ 4/5	CTGCT**ACCGGT**TCTCTCTCSCAGCYTGTGCTGACTCA
5′ AgeI Vλ 6	CTGCT**ACCGGT**TCTTGGGCCAATTTTATGCTGACTCAG
5′ AgeI Vλ 7/8	CTGCT**ACCGGT**TCCAATTCYCAGRCTGTGGTGACYCAG
Reverse	
3′ XhoI Cλ	CTCCTCA**CTCGAG**GGYGGGAACAGAGTG

Continued from table 12

Table 13: Ig Insert Check PCR Primers[46]

***IgH* Insert Check PCR**	
Primer	**Sequence**
Forward	
5’ Ab sense	GCTTCGTTAGAACGCGGCTAC
Reverse	
3’IgGinternal	GTTCGGGGAAGTAGTCCTTGAC
***Igk* Insert Check PCR**	
Primer	**Sequence**
Forward	
5’ Ab sense	GCTTCGTTAGAACGCGGCTAC
Reverse	
3’Cκ494	GTGCTGTCCTTGCTGTCCTGCT
***Igλ* Insert Check PCR**	
Primer	**Sequence**
Forward	
5’ Ab sense	GCTTCGTTAGAACGCGGCTAC
Reverse	
3’Cλ	CACCAGTGTGGCCTTGTTGGCTTG

Table 14: Ig Sequencing Primers[46]

Ig Sequencing Primers	
IgH	
3' CmCH1	GGGAATTCTCACAGGAGACGA
hCh-al-inner	GTCCGCTTTCGCTCCAGGTCACACT
IgG-internal	GTTCGGGGAAGTAGTCCTTGAC
Igk	
3' Ck494	GTGCTGTCCTTGCTGTCCTGCT
Igλ	
hCl-040	TCAGAGGAGGGYGGGAACAGAGTG

Strategy to clone and express human monoclonal antibodies

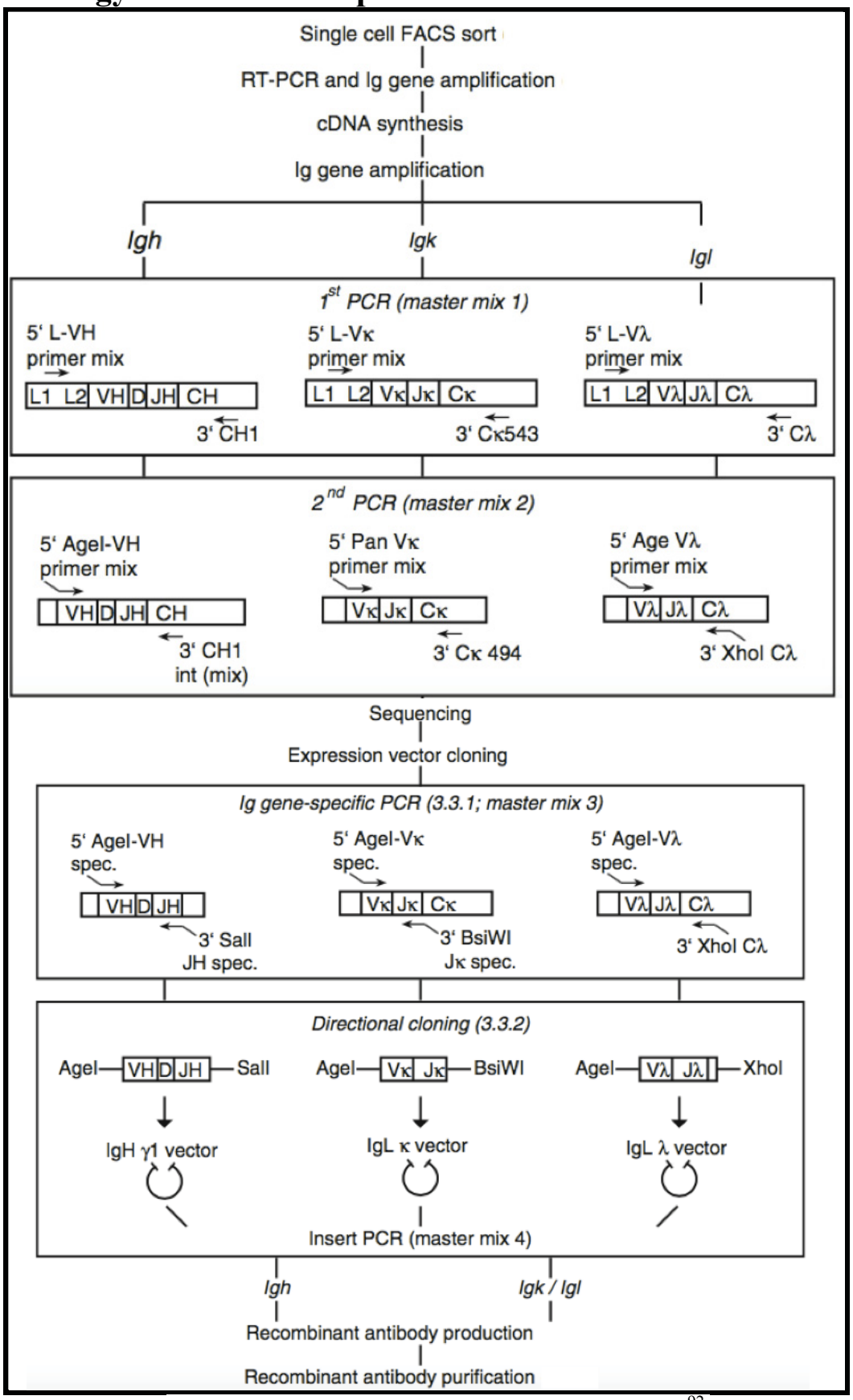
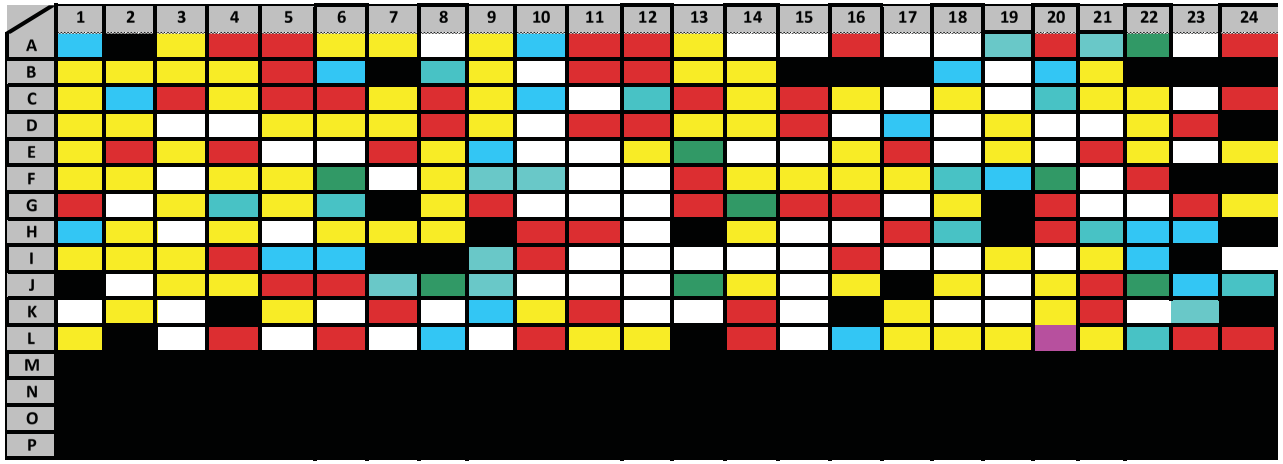


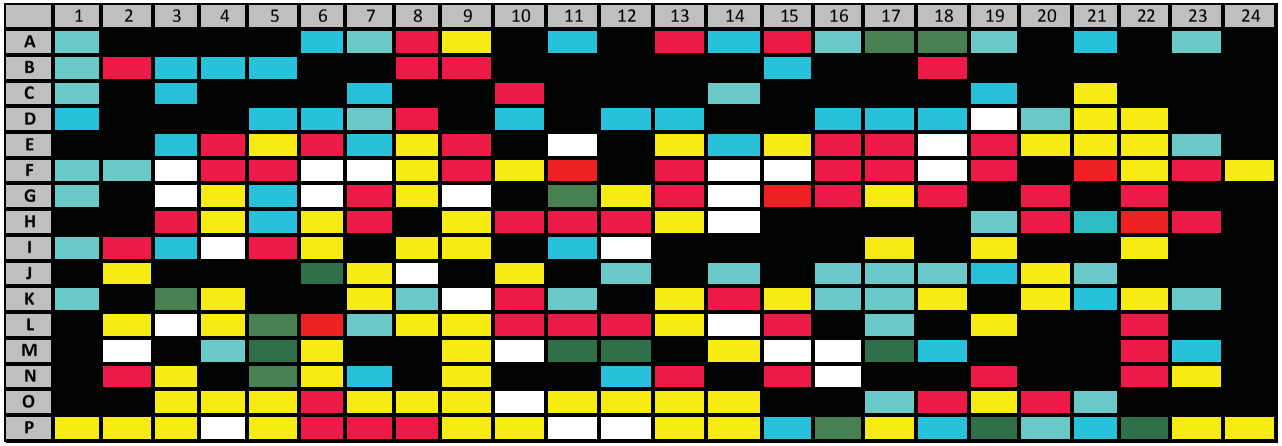
Fig 16: Strategy to clone and express human monoclonal antibodies [92].

Using random hexamer primers (Primer Sequence: 5´ – d (NNNNNN) –3´ N = G, A, T or C), an initial RT-PCR was run to copy the single B cell transcriptome of each sorted cell. A three-way PCR system was employed to amplify heavy, kappa and/or lambda chains of each sorted plasmablast by running heavy, kappa and lambda chain PCR on a fraction (volume) of the cDNA product of each plasmablast. To copy Ig genes from the generated cDNA pool, a nested PCR system was set up: primary PCR with forward primer(s) copying Ig genes from the leader region and reverse primer(s) copying Ig genes from the corresponding constant region of the Ig chain. Secondary PCRs are performed with forward primer(s) specific for the framework region one (FWR1) and reverse primers specific for the corresponding constant region of the Ig chain [*see above for primer sequences and Wardemann et al, 2003, Tiller et al 2008 and Murugan et al, 2015 for comprehensive single B cell PCR method [41, 42, 72]*]. Secondary PCR products from cells with corresponding heavy and light chain genes successfully amplified in PCR and identified on agarose gel were further processed by sequencing a fraction of the PCR product *[see materials and methods for sequencing details]*.

I



II



III

VACCINEES	BLACK	BLUE	TURQUOISE	GREEN	MAGENTA	RED	YELLOW	WHITE
Vax2	8.9	8.3	4.7	2.6	0.0	20.8	31.3	23.4
Vax3	34.4	9.4	9.1	3.9	0.0	16.1	19.5	7.6
Vax4	10.4	7.3	6.3	3.1	1.0	16.7	23.9	31.3

black = **Failed PCR in both heavy and light chains**

blue = **Only lambda chain PCR amplicons**

Turquoise = **Only kappa chain PCR amplicons**

Green = **Only kappa & lambda chain PCR amplicons**

magenta = **Only heavy chain PCR amplicons**

= **Both heavy and lambda chain PCR amplicons**

yellow = **Both heavy and kappa chain PCR amplicons**

white = **Heavy, Kappa & Lambda chain PCR amplicons**

Fig. 7: Single B cell Ig Gene PCR Amplification: (I) Vax2 plasmablast: Row A – H and Vax4 plasmablast: Row I – L **(II)** Complete plate of Vax3 plasmablast. **(III)** Frequencies of Ig gene PCR amplification efficiency from (I) and (II). NB: Each box represents a single cell sorted into a corresponding well on the 384-well plate.

Table 15: Vax3 Ig Sequence D

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N6PMPB091	IGHV4-39*07	IGHD6-13*01	IGHJ4*01	SRVIAAASFDY	11	3	IGHG2	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	6	IGKC	IGLV1-40*01	IGLJ1*01	QSYDSLSGYV	24	IGLC1
N6PMPB114	IGHV3-23*04	IGHD3-3*02	IGHJ2*01	DPFLIRVAGDWYFDL	15	19	IGHA1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	12	----
N6PMPB123	IGHV1-8*01	IGHD7-27*01	IGHJ3*01	GYWGAFDV	8	12	IGHA2	IGKV3-20*01	IGKJ1*01	QQYGSSPRT	7	IGKC					
N6PMPB126	IGHV3-64*01	IGHD3-22*01	IGHJ3*02	GTMTDAFDI	9	1	IGHG1	IGKV2-28*01	IGKJ4*01	MQALQTPT	10	IGKC	IGLV1-47*01	IGLJ3*02	ATWDDSLTIV	14	IGLC3
N6PMPB127	IGHV5-51*01	IGHD2-15*01	IGHJ6*02	RRGRYCTGGTCYFDYSMDV	19	9	IGHG3	IGKV2-28*01	IGKJ4*01	MQALQTPT	6	IGKC					
N6PMPB134	IGHV4-59*01	IGHD3-22*01	IGHJ5*02	GPTMIQE	7	1	IGHG3	IGKV1-5*01	IGKJ2*01	QHYKTYSPT	12	IGKC					
N6PMPB135	IGHV3-15*01	IGHD2-15*01	IGHJ4*02	DCTAGDSCSPYFDS	14	22	IGHA1	IGKV1-16*02	IGKJ5*01	QQYSAYPLT	25	IGKC					
N6PMPB138	IGHV3-23*04	IGHD2-21*02	IGHJ2*01	DPFMMRLAGDWYFDL	15	22	IGHG1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	15	IGLC2
N6PMPB147	IGHV4-4*07	IGHD3-10*01	IGHJ6*02	AGAVLTPLWFGETHYYYYPMDV	22	13	IGHG1	IGKV3-20*01	IGKJ2*01	QQYGTSHLYT	12	IGKC	IGLV3-21*01	IGLJ3*02	QVWHTSSDHRGV	26	IGLC3
N6PMPB150	IGHV1-8*01	IGHD3-10*01	IGHJ6*02	DVPPRMSMVRGIITSYPYSGMDV	23	15	IGHA1	IGKV3-20*01	IGKJ1*01	QQYGSSPPRT	12	IGKC	IGLV1-51*01	IGLJ3*02	ATWDSSLSAGV	19	IGLC3
N6PMPB153	IGHV3-15*01	IGHD5-5*01	IGHJ4*02	VFLNDRLLGYTYGWSSVFDY	20	14	IGHG1	IGKV1D-39*01	IGKJ3*01	QQSFSSLGPT	24	IGKC					
N6PMPB158	IGHV4-59*08	IGHD3-10*01	IGHJ5*02	HHTLYGSGKDWFDP	14	20	IGHA2	IGKV1-5*01	IGKJ1*01	QQYSSYWT	21	IGKC	IGLV1-44*01	IGLJ3*02	AAWDDSLNGWV	1	IGLC3
N6PMPB196	IGHV3-21*01	IGHD2-2*03	IGHJ4*02	HFSCRSTTCPPFDY	14	11	IGHA2	IGKV1D-17*02	IGKJ1*01	LQHNSYPRT	3	IGKC					
N6PMPB224	IGHV4-59*08	IGHD2-2*02	IGHJ3*02	RWEDIVVVPAALAVGSVSYADAFDI	25	1	IGHG1	IGKV1D-39*01	IGKJ5*01	QQSYTTPIT	5	IGKC					
N6PMPB267	IGHV3-23*04	IGHD3-9*01	IGHJ2*01	DPFMMRLAGDWYFDL	15	24	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDTSLGGYVL	11	----
N6PMPB298	IGHV3-11*01	IGHD3-3*01	IGHJ6*02	DQAALKNFDFWSAYPAPQYYFGMA	25	26	IGHG4	IGKV3-15*01	IGKJ2*01	QHYNNWPYT	10	IGKC					
N6PMPB299	IGHV3-23*04	IGHD2-21*02	IGHJ1*01	GLDCGGDCWSY	11	7	----	IGKV1-16*02	IGKJ5*01	QQYNSYPIT	10	IGKC					
N6PMPB303	IGHV1-8*01	IGHD3-10*01	IGHJ6*02	AWDVEDREDFGMDV	14	23	IGHA1	IGKV1D-39*01	IGKJ1*01	QQSSTTPWT	18	IGKC					
N6PMPB304	IGHV3-23*04	IGHD4-23*01	IGHJ4*02	IRWSVLKDLDY	11	11	IGHG1	IGKV3-11*01	IGKJ4*01	QQRSDWPLT	16	IGKC	IGLV3-10*01	IGLJ3*01	YSTDSSDTYVV	10	IGLC2
N6PMPB328	IGHV3-73*01	IGHD3-16*02	IGHJ4*01	VTPFGFDY	8	30	IGHG1	IGKV3-11*01	IGKJ4*01	QQRSDWPLT	16	IGKC	IGLV1-40*02	IGLJ3*01	QSYDSSLSVV	3	IGLC2
N6PMPB346	IGHV1-46*01	IGHD3-16*02	IGHJ4*02	DSHDYVWGRFDY	12	11	IGHG1	IGKV1D-43*01	IGKJ4*01	QQYYSTPQLT	9	IGKC	IGLV1-40*02	IGLJ3*01	QSYDSSLGGYVV	11	----
N6PMPB371	IGHV3-23*04	IGHD3-16*01	IGHJ2*01	DPFLIRLGGDYYFDL	15	36	IGHG1										
N6PMPB372	IGHV3-64*01	IGHD2-8*02	IGHJ4*02	GVYLSQLINNDVFDY	15	23	----	IGKV3-20*01	IGKJ1*01	QQYGTSPPYT	11	IGKC					
N6PMPB069	IGHV3-74*01	IGHD3-10*01	IGHJ5*02	DSGGPNAYNWFDS	13	16	IGHG2	IGKV3-20*01	IGKJ4*01	QQYGTSPLT	5	IGKC					
N6PMPB101	IGHV3-11*01	IGHD3-3*01	IGHJ4*02	DRYDFWSGIFDY	12	6	IGHG1	IGKV3-11*01	IGKJ4*01	HQRSNWPPT	5	IGKC					
N6PMPB104	IGHV3-9*01	IGHD1-26*01	IGHJ3*02	AEGATLGAFDL	11	12	IGHA1	IGKV3-20*01	IGKJ1*01	QQFGRSPQT	14	IGKC					
N6PMPB117	IGHV3-13*01	IGHD2-8*02	IGHJ6*02	DQSTGAGTGV	10	2	IGHG1	IGKV1-39*01	IGKJ1*01	QQSYSTPRT	4	IGKC					
N6PMPB118	IGHV1-46*01	IGHD6-19*01	IGHJ5*02	GIDSTVWQIH	10	24	IGHA1	IGKV1D-39*01	IGKJ2*01	QQSYSIPYT	15	IGKC					
N6PMPB128	IGHV4-34*06	IGHD5-24*01	IGHJ4*02	DRGPNYGYELGY	12	52	IGHA1	IGKV3-20*01	IGKJ4*01	QQFARSRT	27	IGKC					
N6PMPB142	IGHV3-23*04	IGHD3-10*02	IGHJ4*02	GTLTLVRGWLDY	12	21	IGHA1	IGKV1-5*01	IGKJ1*01	QQYKSYSSKT	13	IGKC					
N6PMPB148	IGHV4-39*07	IGHD2-2*03	IGHJ4*02	SRVVAAAMFDY	11	18	IGHG1	IGKV1-33*01	IGKJ1*01	QQFDTLPGT	11	IGKC					
N6PMPB161	IGHV4-39*07	IGHD6-6*01	IGHJ4*02	SRVIGAGSFDY	11	3	IGHG1	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	0	IGKC					
N6PMPB174	IGHV3-23*04	IGHD2-21*01	IGHJ6*04	DRGKERRPKQTGKSYYYYYGLDV	23	2	IGHA1	IGKV3-15*01	IGKJ2*02	QQYNNWPLYT	6	IGKC					
N6PMPB177	IGHV3-9*01	IGHD3-10*01	IGHJ6*02	DRVTMVRGYGMDV	13	2	IGHG2	IGKV4-1*01	IGKJ2*01	QQHHSTPYT	12	IGKC					
N6PMPB181	IGHV4-39*07	IGHD3-10*02	IGHJ4*02	GTSFHYGSGSFYKQPPFDS	19	33	IGHG1	IGKV4-1*01	IGKJ4*01	QQYYNTPLT	9	IGKC					
N6PMPB198	IGHV4-39*01	IGHD3-3*02	IGHJ4*02	RPFSALLEWLFTPFDN	16	17	IGHG4	IGKV1-5*03	IGKJ3*01	QQYNSYLFT	14	IGKC					
N6PMPB201	IGHV4-31*03	IGHD4-17*01	IGHJ4*02	TTTVTPNFDY	10	8	IGHA1	IGKV1D-39*01	IGKJ1*01	QQSYSMWWT	9	IGKC					
N6PMPB211	IGHV1-69*09	IGHD4-17*01	IGHJ3*02	DPGGQADDGDYEGAFDI	17	7	IGHG1	IGKV4-1*01	IGKJ1*01	QQYYSTPwT	4	IGKC					
N6PMPB214	IGHV3-11*01	IGHD5-5*01	IGHJ5*02	GHSYGSF	7	19	IGHM	IGKV3-20*01	IGKJ5*01	QRYGSSLGT	18	IGKC					
N6PMPB218	IGHV3-23*04	IGHD2-21*02	IGHJ5*02	GDCPFTTCHRVPR	13	46	IGHA2	IGKV1-13*02	IGKJ4*01	QQFKSFPYT	26	IGKC					
N6PMPB223	IGHV1-69*01	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	21	IGHG1	IGKV1D-39*01	IGKJ1*01	QQGYSTPPWT	33	IGKC					
N6PMPB226	IGHV3-23*04	IGHD3-22*01	IGHJ4*02	NPTYYYDSSGYGGYFDY	18	2	IGHG1	IGKV1-13*01	IGKJ2*01	QQFNSYLYT	4	IGKC					
N6PMPB247	IGHV4-59*01	IGHD1-26*01	IGHJ4*02	GGGWDLLDY	9	19	IGHG2	IGKV1D-39*01	IGKJ3*01	QQSGTTPL	13	IGKC					
N6PMPB253	IGHV3-15*01	IGHD3-22*01	IGHJ5*02	HHYDRSGYHPPAT	13	42	----	IGKV3-20*01	IGKJ4*01	HHYGNSPPLT	25	IGKC					
N6PMPB255	IGHV3-33*01	IGHD3-22*01	IGHJ4*02	DRNYDSSGYLVSYFDY	16	5	IGHG1	IGKV1D-16*01	IGKJ4*01	QQYDSYPLT	2	IGKC					
N6PMPB258	IGHV3-30*04	IGHD3-22*01	IGHJ4*02	DREWLLLHY	9	2	IGHG1	IGKV1-16*02	IGKJ5*01	QQYNSYPIT	4	IGKC					
N6PMPB260	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	GEFGELHSHFDY	12	17	IGHM	IGKV2-30*01	IGKJ1*01	MQGTHWPLT	11	IGKC					
N6PMPB262	IGHV3-7*01	IGHD5-12*01	IGHJ4*02	DIRGGTYFDY	10		IGHG1	IGKV3-15*01	IGKJ4*01	QQYNDWPLT	4	IGKC					
N6PMPB268	IGHV3-33*01	IGHD1-26*01	IGHJ4*02	IGRVGATPFDY	11	2	IGHM	IGKV3-15*01	IGKJ2*01	QQHNNWPPYT	1	IGKC					
N6PMPB272	IGHV4-31*03	IGHD6-19*01	IGHJ6*02	DYRVAGKRGGLDV	13	41	IGHG1	IGKV3-20*01	IGKJ5*01	QRSGTSLIT	20	IGKC					
N6PMPB273	IGHV3-7*01	IGHD3-22*01	IGHJ4*02	DYCYESSGHAVLYDY	15	15	----	IGKV2-30*01	IGKJ1*01	QHYGSSPRFT	7	IGKC					
N6PMPB277	IGHV3-11*01	IGHD3-3*01	IGHJ4*02	DPTFTIFGVVIPRQPGGRVVGSLDY	26	21	IGHG1	IGKV1-33*01	IGKJ4*01	QQYHDLLS	13	IGKC					

Table 15 (Continued)

N6PMPB283	IGHV2-26*01	IGHD5-24*01	IGHJ4*02	HSWVELWFDS	10	4	IGHA1		IGKV1-5*01	IGKJ2*02	QHYNTYSPT	24	IGKC							
N6PMPB294	IGHV1-8*01	IGHD7-27*01	IGHJ1*01	AVHTNYMGQVLYSLDS	16	18	----		IGKV1-39*01	IGKJ4*01	QQSYLTPFT	18	IGKC							
N6PMPB297	IGHV1-3*01	IGHD2-2*02	IGHJ4*02	GYCSSTSCQYYFDY	14	20	----		IGKV4-1*01	IGKJ2*01	QQYYSIPYT	13	IGKC							
N6PMPB302	IGHV3-7*01	IGHD3-9*01	IGHJ4*02	GGQAWIQLWYFDY	13	10	IGHG1		IGKV1-6*01	IGKJ3*01	LQDYNYPFT	10	IGKC							
N6PMPB318	IGHV3-23*04	IGHD2-21*02	IGHJ4*02	VPQVTAIHLVDS	13	34	IGHA1		IGKV1-5*03	IGKJ4*02	QRYDSYSGT	26	IGKC							
N6PMPB321	IGHV1-69*09	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	19	IGHG1		IGKV4-1*01	IGKJ2*01	QQCYSVPYT	29	IGKC							
N6PMPB339	IGHV3-7*01	IGHD2-2*03	IGHJ5*02	AGVGYGAF	8	37	IGHA1		IGKV3D-15*01	IGKJ4*01	QQYSNWPLT	26	IGKC							
N6PMPB340	IGHV4-34*02	IGHD3-16*02	IGHJ4*02	GRLPLRGGFTTTFLSRSQTRYFDY	24	20	IGHG3		IGKV3-20*01	IGKJ2*01	QQYGSSPPYT	7	IGKC							
N6PMPB343	IGHV3-21*01	IGHD6-19*01	IGHJ6*02	DNIAVPGSFFALDV	14	12	IGHG2		IGKV3-20*01	IGKJ3*01	QHYGSSPRFT	16	IGKC							
N6PMPB344	IGHV1-69*09	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	20	IGHG1		IGKV4-1*01	IGKJ2*01	QQHHSTPYT	8	IGKC							
N6PMPB345	IGHV4-34*02	IGHD3-9*01	IGHJ6*02	RRNWYYGMDA	10	34	IGHG3		IGKV1-5*03	IGKJ2*01	QQYNRYSFHT	18	IGKC							
N6PMPB349	IGHV5-51*01	IGHD3-3*02	IGHJ5*02	RAYGGSELATNNWFDP	16	36	IGHG2		IGKV2-30*01	IGKJ1*01	MQGSRWPWA	32	IGKC							
N6PMPB350	IGHV3-21*01	IGHD6-13*01	IGHJ4*02	AAAGIYYFDY	10	16	IGHG2		IGKV3-20*01	IGKJ2*01	QQYGSSSRYT	13	IGKC							
N6PMPB355	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	10	IGHG1		IGKV1-5*01	IGKJ2*02	QQYNGYSPT	13	IGKC							
N6PMPB361	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1		IGKV1-5*01	IGKJ2*02	QHYKTYSPT	16	IGKC							
N6PMPB365	IGHV3-23*04	IGHD5-24*01	IGHJ4*01	ALYNDRWFFDY	11	31	IGHA1		IGKV3D-11*01	IGKJ4*01	QQRGNWPLT	13	IGKC							
N6PMPB369	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIELWFDY	10	6	IGHG1		IGKV1-5*01	IGKJ2*02	QHYNTYSPT	6	IGKC							
N6PMPB370	IGHV3-33*01	IGHD2-2*03	IGHJ6*02	DLWDIVVGSQGDCYYGMDV	19	3	IGHA1		IGKV1D-39*01	IGKJ1*01	QQSYSAPEVT	6	IGKC							
N6PMPB373	IGHV1-69*09	IGHD3-3*01	IGHJ5*02	SLKDYYDFSWFDP	13	5	IGHG2		IGKV4-1*01	IGKJ4*01	QQYYSPPLT	7	IGKC							
N6PMPB374	IGHV1-8*01	IGHD3-22*01	IGHJ4*02	GVSSRGMCYFRD	12	14	----		IGKV1-5*03	IGKJ1*01	QQYNSYSPWT	10	IGKC							
N6PMPB377	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1		IGKV1-5*01	IGKJ2*02	QHYKTYSPT	12	IGKC							
N6PMPB384	IGHV1-69*04	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	18	IGHG1		IGKV4-1*01	IGKJ4*01	QHYYSPPLG	19	IGKC							
N6PMPB008	IGHV3-23*04	IGHD1-26*01	IGHJ2*01	DPFLIRLAGDYYFDL	15	22	IGHG1							IGLV1-40*02	IGLJ3*01	QSYDTSLGGYVL	12	IGLC2		
N6PMPB013	IGHV3-66*04	IGHD4-17*01	IGHJ6*02	SIATVTTTVDYYYYGMDV	18	6	IGHA1							IGLV3-25*02	IGLJ3*02	QSQDTSGTYGV	5	IGLC2		
N6PMPB015	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DQNPLLWFGGSLDY	14	10	IGHG4							IGLV1-44*01	IGLJ3*01	AAWDDSLNAHR	1	IGLC7		
N6PMPB033	IGHV7-4-1*02	IGHD3-22*01	IGHJ6*02	IYHHDSGGPDYHGMDV	16	19	IGHA1							IGLV3-25*02	IGLJ3*02	QSADSSGTYWV	19	IGLC7		
N6PMPB100	IGHV3-73*01	IGHD3-16*02	IGHJ4*01	VTPFGFDY	8	26	IGHG1							IGLV3-10*01	IGLJ3*01	YSTDSSDTYVV	8	IGLC2		
N6PMPB102	IGHV4-39*01	IGHD1-1*01	IGHJ4*02	HLGYNYGHLVY	11	63	IGHM							IGLV2-8*01	IGLJ3*01	TSYAGNNILV	6	IGLC2		
N6PMPB105	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	SGGWWAFDY	9	22	----							IGLV2-8*01	IGLJ3*02	SSYGGSANVL	10	IGLC7		
N6PMPB112	IGHV3-15*01	IGHD6-25*01	IGHJ6*02	GVGMGGMDV	9	6	IGHG1							IGLV9-49*01	IGLJ3*02	GADHGSGSNFFW	2	IGLC7		
N6PMPB113	IGHV3-11*01	IGHD3-22*01	IGHJ6*02	NRGGFYIYGMDV	12	17	IGHG1							IGLV6-57*01	IGLJ3*01	QSYDSSDVV	9	----		
N6PMPB115	IGHV4-59*01	IGHD6-25*01	IGHJ4*02	ESGIAAAVSYFDS	13	20	IGHA1							IGLV3-10*01	IGLJ3*01	YSTDTSGNHRV	5	IGLC7		
N6PMPB124	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DRLKYYGSGSPLDY	14	8	IGHG1							IGLV3-21*01	IGLJ3*02	QVWHTSSDHRGV	8	IGLC2		
N6PMPB125	IGHV4-31*01	IGHD6-19*01	IGHJ5*02	GLSGNYYVGWFDP	13	28	IGHA2							IGLV3-25*02	IGLJ3*01	QSADSSGFYVV	22	IGLC2		
N6PMPB129	IGHV3-66*04	IGHD3-22*01	IGHJ6*02	NIYYDAGTADHYYYGMDV	18	12	IGHG1							IGLV3-25*02	IGLJ3*02	QSQDSSGTIGV	27	IGLC7		
N6PMPB137	IGHV3-33*01	IGHD3-9*01	IGHJ3*02	LSGTPSVYETSTDAFDI	17	38	IGHA1							IGLV3-10*01	IGLJ1*01	YSADTSGHYRGV	22	IGLC1		
N6PMPB159	IGHV3-13*01	IGHD6-6*01	IGHJ4*02	GLAAAQFDY	9	1	IGHG1							IGLV4-60*03	IGLJ3*02	ETWDSNTRV	6	IGLC7		
N6PMPB162	IGHV3-9*01	IGHD3-10*01	IGHJ6*02	DTSPLWWFGDQDNYGMDV	18	5	IGHG1							IGLV1-47*01	IGLJ3*02	AAWDDSLSGYWV	2	IGLC7		
N6PMPB166	IGHV3-7*01	IGHD3-16*02	IGHJ4*02	LLGLGEVSPYFDF	13	24	----							IGLV1-44*01	IGLJ3*01	AAWDGSLNIPL	12	IGLC2		
N6PMPB171	IGHV3-9*01	IGHD3-10*01	IGHJ3*02	DTTPLVWFGELLGDDAFDI	19	3	IGHG1							IGLV1-41*01	IGLJ3*02	AAWDDSLNGWV	1	IGLC7		
N6PMPB175	IGHV3-9*01	IGHD3-10*01	IGHJ6*02	DRVTMVRGYGMDV	13	2	IGHG2							IGLV1-40*01	IGLJ3*01	QSYDSSLSGSYVL	14	----		
N6PMPB179	IGHV3-7*01	IGHD3-3*01	IGHJ4*02	APQYFDFWSSDY	12	2	IGHG1							IGLV3-1*01	IGLJ3*01	QAWDSSTVI	2	IGLC7		
N6PMPB190	IGHV3-33*01	IGHD3-22*01	IGHJ4*02	RGGGYYDSSGYDGSFDY	17	4	IGHG2							IGLV3-25*02	IGLJ3*02	QSADSSGSWV	5	IGLC7		
N6PMPB194	IGHV3-23*04	IGHD1-26*01	IGHJ3*02	GSEWELPDAFDI	12	1	IGHG1							IGLV3-25*02	IGLJ3*02	QSADSSGTHKV	2	IGLC7		
N6PMPB250	IGHV3-11*01	IGHD3-16*02	IGHJ5*02	GRGSYLPPGLDH	12	22	IGHA2							IGLV3-21*02	IGLJ3*01	QVWDSNRQHPYM	20	IGLC7		
N6PMPB274	IGHV3-73*01	IGHD3-16*02	IGHJ4*01	VTPFGFDY	8	31	IGHG1							IGLV3-10*01	IGLJ3*01	YSTDSSDTYVV	8	IGLC2		
N6PMPB275	IGHV3-43*01	IGHD6-13*01	IGHJ4*02	GEQQLVPTPGVFDY	14	7	IGHG2							IGLV3-25*02	IGLJ3*02	QSADSSSTYEV	4	IGLC7		
N6PMPB276	IGHV3-30*19	IGHD3-22*01	IGHJ6*02	DQYANWKSAYYYYYGMEV	18	22	IGHA1							IGLV2-23*02	IGLJ3*02	CSYAGTNTFV	14	----		
N6PMPB279	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DSYGSGIYRRMDN	13	35	IGHA1							IGLV2-11*01	IGLJ1*01	CSFAGKFTYV	24	IGLC1		
N6PMPB314	IGHV3-49*03	IGHD2-2*03	IGHJ6*02	YKLVVVPGILHGGYHRGMDV	20	21	IGHA1							IGLV2-14*01	IGLJ3*01	NSYTTSSTLVL	18	----		
N6PMPB325	IGHV3-72*01	IGHD2-21*02	IGHJ4*02	LSGSY	5	15	IGHM							IGLV1-47*01	IGLJ3*02	AVWDDSLSGRV		IGLC2		
N6PMPB331	IGHV3-53*01	IGHD2-21*02	IGHJ5*02	HPFRR*NYCGDHDCLDP	17	27	IGHG2							IGLV2-8*01	IGLJ3*02	SSYAGNLEL	14	IGLC2		
N6PMPB334	IGHV3-7*01	IGHD1-7*01	IGHJ3*02	FTENYRAFDL	10	16	----							IGLV2-11*01	IGLJ3*02	CSYGGSSTWV	9	IGLC3		
N6PMPB342	IGHV3-23*04	IGHD4-23*01	IGHJ6*02	SPNHDILTDYGMDV	14	11	IGHG1							IGLV3-25*02	IGLJ3*02	QSADSSGTYQV	17	IGLC3		
N6PMPB354	IGHV3-9*01	IGHD1-20*01	IGHJ4*02	DISPGTTALSILFDY	15	2	IGHG1							IGLV1-44*01	IGLJ3*02	AAWDDSLGV	0	IGLC3		
N6PMPB356	IGHV3-11*01	IGHD4-17*01	IGHJ6*02	GTYGDYRQVGMDV	13	23	IGHA2							IGLV1-47*01	IGLJ3*01	ATWDDSLGGL	16	----		
N6PMPB367	IGHV3-33*04	IGHD4-4*01	IGHJ6*02	DDVNSNFLGGWPSMDV	16	16	IGHG1							IGLV3-1*01	IGLJ3*01	QAWDNNHVV	20	IGLC2		
N6PMPB368	IGHV3-11*01	IGHD3-3*01	IGHJ4*02	DGSGYYNPYFDY	12	4	IGHG1							IGLV3-1*01	IGLJ3*01	QAWDSSTVV	2	----		

Table 16: Vax2 Ig Sequences Data

	HEAVY CHAIN							KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N5PMPB110	IGHV3-21*01	IGHD3-3*01	IGHJ3*02	DVQSLITIFGNFDI	14	11	IGHG1	IGKV3-11*01	IGKJ5*01	QQRSNWPLT	6	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB116	IGHV1-69*01	IGHD3-9*01	IGHJ6*03	HVGIRRSFDSIHYYYMDV	18	21	IGHA1	IGKV3-20*01	IGKJ4*02	QQYGVSPL	17	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB127	IGHV1-46*02	IGHD3-10*01	IGHJ6*02	DLTMVRGLVGYYGMDV	16	24	IGHG1	IGKV2-28*01	IGKJ2*01	MQALQTPPYT	6	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB156	IGHV1-46*02	IGHD1-26*01	IGHJ5*02	DYSHTNWLGP	10	30	IGHA1	IGKV3-20*01	IGKJ2*02	QHYDHSPRVYT	23	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB165	IGHV3-49*04	IGHD6-19*01	IGHJ4*02	TTLNAQWPIFDY	12	38	IGHG2	IGKV3-15*01	IGKJ4*01	QQYNKWPPLT	28	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB166	IGHV6-1-01	IGHD1-7*01	IGHJ4*02	MTREALENYFDY	12	16	IGHM	IGKV2-30*01	IGKJ1*01	MQATHWPPT	9	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB003	IGHV3-23*04	IGHD3-22*01	IGHJ4*02	DRYSTASYYFDY	12	31	IGHA1	IGKV1-12*01	IGKJ2*02	QLPNSLPPIYA	24	IGKC					
N5PMPB006	IGHV3-30-3*01	IGHD2-2*02	IGHJ5*02	GGRTYRHRFDP	11	34	IGHG3	IGKV1-39*01	IGKJ4*01	QQSFSSPFT	21	IGKC					
N5PMPB013	IGHV4-34*01	IGHD3-9*01	IGHJ6*03	GRRYFDWPTFVMDV	14	1	IGHM	IGKV1-17*01	IGKJ4*01	LQHNSYPLT	6	IGKC					
N5PMPB014	IGHV1-2*02	IGHD2-2*02	IGHJ4*01	GYSDIIGRCLDY	12	15	IGHA2	IGKV1-9*01	IGKJ1*01	QQLNSYPRT	4	IGKC					
N5PMPB025	IGHV3-21*01	IGHD1-14*01	IGHJ6*03	VANLRANRYYYYYMDV	16	0	IGHM	IGKV3-11*01	IGKJ2*01	QQRSNWLYT	3	IGKC					
N5PMPB027	IGHV4-59*01	IGHD2-15*01	IGHJ6*02	GGYSPKYYYYYGMDV	15	0	IGHM	IGKV3-20*01	IGKJ2*01	QQYGSSLYT	1	IGKC					
N5PMPB033	IGHV4-4*02	IGHD6-19*01	IGHJ3*02	GTKAVPNNDAFDI	13	12	IGHA1	IGKV3-11*01	IGKJ2*01	QHRSNWPQYT	6	IGKC					
N5PMPB037	IGHV3-7*01	IGHD5-24*01	IGHJ4*02	AKHGY	5	0	IGHG1	IGKV3-11*01	IGKJ4*02	QQRSNWPWT	3	IGKC					
N5PMPB038	IGHV4-59*01	IGHD1-7*01	IGHJ5*02	LSSGTSNWFDP	11	2	IGHM	IGKV1-12*01	IGKJ4*01	QQANSFPLS	12	IGKC					
N5PMPB045	IGHV3-7*01	IGHD6-25*01	IGHJ4*02	TYRSSGTDY	9	14	IGHG2	IGKV2-30*01	IGKJ3*01	VQLTQWPFT	7	IGKC					
N5PMPB049	IGHV3-49*04	IGHD3-22*01	IGHJ3*02	DLSRNYYDSSGADAFDI	17	0	IGHM	IGKV1-5*03	IGKJ2*01	QQYNSYPYT	2	IGKC					
N5PMPB052	IGHV3-74*01	IGHD1-26*01	IGHJ4*02	GRRSSGTYEPY	11	27	IGHG1	IGKV2-28*01	IGKJ4*01	MQALRIPFT	15	IGKC					
N5PMPB060	IGHV3-30*18	IGHD3-22*01	IGHJ4*02	EFGNYYETSGHPYYFHY	17	12	IGHM	IGKV1-5*03	IGKJ1*01	QKYNSYSKT	13	IGKC					
N5PMPB062	IGHV1-46*01	IGHD5-24*01	IGHJ5*02	WLKGDETDH	9	13	IGHA1	IGKV1-17*01	IGKJ1*01	LQHDTYPWT	13	IGKC					
N5PMPB073	IGHV3-49*04	IGHD6-19*01	IGHJ6*03	PYSSGWNYYYYYYMDV	16	4	IGHM	IGKV1-16*02	IGKJ3*01	QQYNSYP	8	IGKC					
N5PMPB074	IGHV1-69*06	IGHD3-16*02	IGHJ5*02	DQVPWLGAEVPVALES	16	45	IGHG3	IGKV3-20*01	IGKJ4*01	HQYGDSPLT	25	IGKC					
N5PMPB078	IGHV3-48*03	IGHD5-12*01	IGHJ4*02	DRDSGYVGDYFDY	13	9	IGHM	IGKV3-15*01	IGKJ3*01	QQYNVWPPPVST	10	IGKC					
N5PMPB081	IGHV3-48*02	IGHD6-19*01	IGHJ4*02	SGWSRYHFDH	10	10	IGHG1	IGKV3-20*01	IGKJ1*01	QQYGSSLWT	6	IGKC					
N5PMPB082	IGHV1-58*01	IGHD6-19*01	IGHJ6*01	VGDGAVAGLNYGMDV	15	7	IGHG1	IGKV1-39*01	IGKJ1*01	QQSYSTPWT	4	IGKC					
N5PMPB085	IGHV3-30*14	IGHD3-10*02	IGHJ5*02	DKGNYYTSGSHFRVLDS	17	33	IGHG1	IGKV3-20*01	IGKJ5*01	QQYYDSPPIT	30	IGKC					
N5PMPB088	IGHV3-9*01	IGHD2-2*02	IGHJ1*01	ESPPCTNTWSSFHR	14	22	IGHG2	IGKV3-20*01	IGKJ4*02	HQYGSSPLT	13	IGKC					
N5PMPB090	IGHV1-69*01	IGHD3-16*02	IGHJ2*01	ERGGYDWYFDL	11	11	IGHG1	IGKV3-15*01	IGKJ1*01	HQYNHWGT	13	IGKC					
N5PMPB099	IGHV1-18*01	IGHD4-17*01	IGHJ4*02	DVPTTVTTRERFDY	14	1	IGHM	IGKV1-5*03	IGKJ5*01	QHYNSYPIT	12	IGKC					
N5PMPB104	IGHV4-7-1*02	IGHD4-17*01	IGHJ5*02	GEDYGDFPF	9	12	IGHA1	IGKV1-5*03	IGKJ1*01	QHYKSY	7	IGKC					
N5PMPB112	IGHV3-23*04	IGHD4-17*01	IGHJ3*02	KLHDYVQGGAFDI	13	25	IGHG1	IGKV1-5*03	IGKJ2*01	QQYYSYLYT	14	IGKC					
N5PMPB118	IGHV3-9*01	IGHD3-16*02	IGHJ2*01	NGGGSTWHWYFDL	13	16	IGHG1	IGKV3D-15*01	IGKJ3*01	QQYNNWPLS	5	IGKC					
N5PMPB123	IGHV4-34*01	IGHD4-23*01	IGHJ5*02	EGVRRYGGEPT	11	14	IGHG1	IGKV3-11*01	IGKJ3*01	QQRSNWPPFT	7	IGKC					
N5PMPB128	IGHV3-15*01	IGHD5-24*01	IGHJ4*01	PGGRGGPFSRDGYGFAY	17	16	IGHG1	IGKV1-39*01	IGKJ1*01	QHSYYTPWT	14	IGKC					
N5PMPB131	IGHV3-30*18	IGHD6-19*01	IGHJ4*02	GNAMGPHGCFDY	12	11	IGHG1	IGKV1-39*01	IGKJ4*01	QQSFRTPLT	14	IGKC					
N5PMPB134	IGHV4-34*01	IGHD3-10*01	IGHJ4*02	GRGKLQWFAELYH	13	4	IGHG1	IGKV3-20*01	IGKJ2*01	QQYGSSPRT	5	IGKC					
N5PMPB135	IGHV3-74*03	IGHD2-15*01	IGHJ4*02	GRYCSVGSCYSGQIEN	16	9	IGHA2	IGKV2-28*01	IGKJ4*01	MQALQTPPT	5	IGKC					
N5PMPB136	IGHV3-23*04	IGHD6-19*01	IGHJ5*01	LQSSGWYSDL	10	19	IGHG1	IGKV1-39*01	IGKJ2*01	QQSYNTYT	10	IGKC					
N5PMPB152	IGHV3-23*04	IGHD3-22*01	IGHJ3*02	GGYYDSSGYPNPLDI	15	10	IGHA1	IGKV2-28*01	IGKJ1*01	MQTLQTPWT	9	IGKC					
N5PMPB172	IGHV3-30*18	IGHD3-10*02	IGHJ4*02	EGFTTGWTFGDY	12	32	IGHG3	IGKV1-16*01	IGKJ3*01	QQYKTYPHT	20	IGKC					
N5PMPB182	IGHV1-8*01	IGHD3-16*02	IGHJ5*02	GAGEGGRDWFDP	12	20	IGHA2	IGKV4-1*01	IGKJ4*01	QQYHSKPPT	11	IGKC					
N5PMPB008	IGHV3-30*18	IGHD5-24*01	IGHJ3*01	DVRIAPTITTGSSFDV	16	24	IGHG1	IGKV4-1*01	IGKJ2*01	QQHHSTPYT	8	IGKC					
N5PMPB107	IGHV3-48*02	IGHD5-24*01	IGHJ4*02	DGSVSYPDY	9	15	IGHA1	IGKV3-11*01	IGKJ4*01	QQRTNWPFLLT	18	IGKC					
N5PMPB111	IGHV3-7*01	IGHD6-19*01	IGHJ5*02	RMVAVIGTGTFA	12	17	IGHG1	IGKV4-1*01	IGKJ1*01	LQNYNGRWT	23	IGKC					
N5PMPB141	IGHV1-18*01	IGHD6-19*01	IGHJ5*02	TESRQAGMQSGLDP	14	30	IGHA1	IGKV2-28*01	IGKJ4*01	MQALHTPLT	12	IGKC					
N5PMPB154	IGHV3-23*04	IGHD3-10*02	IGHJ4*02	DLGRVGELLCFDY	13	9	IGHG3	IGKV2-28*01	IGKJ4*02	MQARQIRLT	19	IGKC					
N5PMPB012	IGHV3-9*01	IGHD5-5*01	IGHJ4*02	DIHPLKIQLWLGGFDY	16	7	IGHG1						IGLV2-23*03	IGLJ1*01	CSYAGSSTYV	3	----
N5PMPB029	IGHV1-69*01	IGHD4-17*01	IGHJ5*02	GGWTISTESTRGHLEQ	17	31	IGHA1						IGLV8-61*01	IGLJ3*02	VLYLGSYNWV	13	----
N5PMPB053	IGHV3-74*01	IGHD1-26*01	IGHJ3*02	GTKALPRSTLDL	13	16	IGHM						IGLV2-8*01	IGLJ2*01	SSFARSNSVV	11	IGLC2
N5PMPB054	IGHV5-51*01	IGHD3-10*01	IGHJ6*02	STATMAFDGLDV	13	33	IGHA1						IGLV3-27*01	IGLJ2*01	FSGTDNNLG	23	IGLC7
N5PMPB056	IGHV4-4*07	IGHD2-2*02	IGHJ5*02	GGYFSTSGSNWFDP	15	7	IGHM						IGLV2-8*02	IGLJ1*01	SSYAGSNIYV	1	----
N5PMPB061	IGHV4-34*01	IGHD3-10*02	IGHJ5*02	KRQITLLRGVITSAGWFDP	20	1	IGHG3						IGLV9-49*01	IGLJ2*01	GADHGSGSNFVFVV	3	IGLC1
N5PMPB063	IGHV3-30-3*01	IGHD3-16*02	IGHJ4*02	DDEGEGDPIDY	12	31	IGHA2						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	17	IGLC1

Table 16 (Continued)

N5PMPB095	IGHV3-23*01	IGHD3-10*02	IGHJ5*02	YVRAPYP	9	26	IGHG1						IGLV2-14*01	IGLJ3*02	SSYTTSTTLWV	14	IGLC7
N5PMPB103	IGHV3-23*04	IGHD3-3*02	IGHJ4*02	DPTDYSDTSGYWLESYLDC	19	9	IGHG2						IGLV2-14*04	IGLJ3*02	GSYTSSSPLGV	11	IGLC2
N5PMPB133	IGHV3-21*01	IGHD5-12*01	IGHJ3*02	VQQIAGYDQNDGFDI	16	17	IGHG1						IGLV1-40*01	IGLJ1*01	QSYDSSLSGYV	2	IGLC1
N5PMPB142	IGHV3-43*01	IGHD5-24*01	IGHJ3*01	AESGDGFPYEAFD	13	23	IGHA1						IGLV1-47*01	IGLJ2*01	ATWDDRLSVVL	17	IGLC2
N5PMPB153	IGHV3-43*01	IGHD3-3*01	IGHJ6*02	GGYYDFWSGYDKRPYHYYGMDV	23	5	IGHA1						IGLV2-14*01	IGLJ1*01	SSYTGSSTDV	2	IGLC1
N5PMPB157	IGHV3-33*01	IGHD2-15*01	IGHJ3*02	EGGYCSGGSCSFGYAFDI	19	5	IGHM						IGLV2-8*02	IGLJ3*02	NSYAGSNIVV	7	IGLC7
N5PMPB164	IGHV4-39*01	IGHD4-17*01	IGHJ2*01	HATVTAGGDFDL	13	10	IGHA1						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB015	IGHV4-39*01	IGHD5-5*01	IGHJ4*02	HINTAMVFDY	12	8	IGHM						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	18	IGLC1
N5PMPB017	IGHV3-7*01	IGHD6-6*01	IGHJ4*02	RASEY	6	16	IGHA2						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB023	IGHV3-53*01	IGHD2-15*01	IGHJ4*02	FCSAGTCDSEEAFDY	16	22	IGHA1						IGLV2-23*02	IGLJ1*01	FSYARGDSYV	21	---
N5PMPB065	IGHV4-59*01	IGHD3-16*02	IGHJ5*02	GSPPYVWGSYRDTIKENWLDP	22	23	IGHG1						IGLV1-44*01	IGLJ3*02	AAWDDSLNGWV	11	IGLC7
N5PMPB114	IGHV4-59*02	IGHD3-3*02	IGHJ5*02	GNFWSGYYTPNWFDP	16	17	IGHG3						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N5PMPB146	IGHV3-23*04	IGHD3-10*01	IGHJ4*02	GEGSGSFPDY	11	29	IGHG1						IGLV1-47*01	IGLJ2*01	GVWDDRLSGPL	25	IGLC2
N5PMPB155	IGHV5-51*01	IGHD2-2*02	IGHJ4*02	HRSGYCSITSCSHFDY	16	1	IGHG1						IGLV2-23*03	IGLJ1*01	CSYAGSSTNYV	0	---
N5PMPB180	IGHV4-59*01	IGHD5-5*01	IGHJ5*02	GGGYFDP	8	30	IGHG3						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1

Table 17: Vax4 Ig Sequence Data

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N7PMPB206	IGHV3-30*18	IGHD3-10*02	IGHJ5*02	DRYPGEILIWFDP	13	7	IGHA1	IGKV2D-26*01	IGKJ3*01	MQDSQDPLFT	10	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	8	IGLC1
N7PMPB231	IGHV3-49*05	IGHD1-26*01	IGHJ5*02	TIVGVTTAFDL	11	15	IGHA2	IGKV2-28*01	IGKJ4*01	MQALQTPLT	2	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	17	IGLC1
N7PMPB235	IGHV3-64*01	IGHD2-15*01	IGHJ4*02	GGYCSGGSCYLPY	13	0	IGHG1	IGKV4-1*01	IGKJ1*01	QQYYSTPQT	1	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N7PMPB241	IGHV3-30*18	IGHD2-2*02	IGHJ5*02	DRYPGEILIWFDP	13	6	IGHA1	IGKV2D-26*01	IGKJ3*01	MQDSQDPLFT	8	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	7	IGLC1
N7PMPB255	IGHV3-74*03	IGHD3-10*02	IGHJ3*02	DRGWNALDL	9	14	IGHA2	IGKV2-29*02	IGKJ3*01	MQGLHLPLT	11	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N7PMPB258	IGHV4-59*01	IGHD3-9*01	IGHJ4*02	GTPSYDILTGYETAYFDY	18	0	IGHM	IGKV3-20*01	IGKJ5*01	QQYGSSPIT	4	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N7PMPB230	IGHV3-43*01	IGHD3-16*02	IGHJ2*01	DYAYRGYWYFDL	12	23	IGHA1	IGKV1-12*01	IGKJ1*01	QQARSFPWT	17	IGKC					
N7PMPB236	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	18	IGKC					
N7PMPB242	IGHV5-10-1*01	IGHD1-7*01	IGHJ4*03	VPWYNSSTWFAPNW	14	22	IGHA2	IGKV1-16*02	IGKJ4*01	QQYKSYPET	11	IGKC					
N7PMPB245	IGHV3-74*01	IGHD6-19*01	IGHJ4*02	DTRSGWYGGFDY	12	0	IGHM	IGKV4-1*01	IGKJ2*01	QQYYSTPYT	1	IGKC					
N7PMPB252	IGHV3-23*01	IGHD4-11*01	IGHJ5*02	TGQFDY	6	30	IGHM	IGKV2-30*01	IGKJ1*01	VQGSHWPRT	12	IGKC					
N7PMPB265	IGHV3-23*04	IGHD1-14*01	IGHJ4*02	KIAGRNPFDY	10	25	IGHA1	IGKV3-15*01	IGKJ4*01	QQYHDWPPLT	15	IGKC					
N7PMPB275	IGHV3-7*03	IGHD1-7*01	IGHJ2*01	HFWTFGL	7	13	IGHA2	IGKV4-1*01	IGKJ4*01	QQYYSAPLT	11	IGKC					
N7PMPB276	IGHV4-39*01	IGHD6-19*01	IGHJ4*02	RLAVGWPRDYFDY	13	24	IGHG1	IGKV1-12*01	IGKJ1*01	QQAHSFPWT	12	IGKC					
N7PMPB281	IGHV5-51*01	IGHD6-13*01	IGHJ3*02	VRVAAAGRGMYVGNAFDI	18	0	IGHM	IGKV3-20*01	IGKJ3*01	QQYGSSPLFT	4	IGKC					
N7PMPB283	IGHV5-51*01	IGHD6-19*01	IGHJ4*02	RFVGYSSRWPLDS	13	4	IGHG1	IGKV3-15*01	IGKJ2*02	QQYNNWPQT	5	IGKC					
N5PMPB008	IGHV3-30*18	IGHD5-24*01	IGHJ3*01	DVRIAPTITTGSSFDV	16	24	IGHG1	IGKV4-1*01	IGKJ2*01	QQHHSTPYT	8	IGKC					
N5PMPB107	IGHV3-48*02	IGHD5-24*01	IGHJ4*02	DGSVSYPDY	9	15	IGHA1	IGKV3-11*01	IGKJ4*01	QQRTNWPFLLT	18	IGKC					
N5PMPB111	IGHV3-7*01	IGHD6-19*01	IGHJ5*02	RMVAVIGTGTFA	12	17	IGHG1	IGKV4-1*01	IGKJ1*01	LQNYNGRWT	23	IGKC					
N5PMPB141	IGHV1-18*01	IGHD6-19*01	IGHJ5*02	TESRQAGMQSGLDP	14	30	IGHA1	IGKV2-28*01	IGKJ4*01	MQALHTPLT	12	IGKC					
N5PMPB154	IGHV3-23*04	IGHD3-10*02	IGHJ4*02	DLGRVGELLCFDY	13	9	IGHG3	IGKV2-28*01	IGKJ4*02	MQARQIRLT	19	IGKC					
N7PMPB207	IGHV4-39*02	IGHD3-10*01	IGHJ4*02	HSRRFGEFDY	10	37	IGHM	IGKV1-12*01	IGKJ2*01	QQAMSFPYT	18	IGKC					
N7PMPB216	IGHV4-4*02	IGHD3-16*02	IGHJ3*02	VDLGAFDI	8	20	IGHG2	IGKV3-20*01	IGKJ1*01	QQYGRSPWT	11	IGKC					
N7PMPB248	IGHV4-30-4*01	IGHD3-3*02	IGHJ6*02	NAGDNLFYTYRYGLGV	16	17	IGHG1	IGKV3-20*01	IGKJ1*01	QQYGTSAP	12	IGKC					
N7PMPB262	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	13	IGKC					
N7PMPB196	IGHV1-18*01	IGHD6-13*01	IGHJ6*02	EGGVPPEGQQLHILRGYYYYGMDV	25	1	IGHG1						IGLV1-36*01	IGLJ3*02	AAWDDSLNGPV	2	IGLC7
N7PMPB237	IGHV4-30-2*01	IGHD3-22*01	IGHJ2*01	GDFYDTSGYRLTGHWHFDL	19	26	IGHA1						IGLV2-8*02	IGLJ2*01	SSFVGSTTLL	32	IGLC7
N7PMPB261	IGHV3-23*01	IGHD1-1*01	IGHJ6*02	EYRLLTTATHSMDV	14	12	IGHA2						IGLV2-14*01	IGLJ1*01	FSYTITSTPYV	8	IGLC1
N7PMPB270	IGHV3-21*01	IGHD4-17*01	IGHJ3*02	GSTTAQRADLFDI	14	12	IGHA1						IGLV1-44*01	IGLJ3*01	AAWDDSLHGWV	2	IGLC2
N7PMPB287	IGHV3-30*18	IGHD6-19*01	IGHJ5*02	GQFREAGTTTRRWLDS	17	35	IGHG1						IGLV3-21*01	IGLJ3*02	QVWHTSSDHRGV	8	----
N5PMPB015	IGHV4-39*01	IGHD5-5*01	IGHJ4*02	HINTAMVFDY	12	8	IGHM						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	18	IGLC1
N5PMPB017	IGHV3-7*01	IGHD6-6*01	IGHJ4*02	RASEY	6	16	IGHA2						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB023	IGHV3-53*01	IGHD2-15*01	IGHJ4*02	FCSAGTCDSEEAFDY	16	22	IGHA1						IGLV2-23*02	IGLJ1*01	FSYARGDSYV	21	----
N5PMPB065	IGHV4-59*01	IGHD3-16*02	IGHJ5*02	GSPPYVWGSYRDTIKENWLDP	22	23	IGHG1						IGLV1-44*01	IGLJ3*02	AAWDDSLNGWV	11	IGLC7
N5PMPB114	IGHV4-59*02	IGHD3-3*02	IGHJ5*02	GNFWSGYYTPNWFDP	16	17	IGHG3						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N5PMPB146	IGHV3-23*04	IGHD3-10*01	IGHJ4*02	GEGSGSFPDY	11	29	IGHG1						IGLV1-47*01	IGLJ2*01	GVWDDRLSGPL	25	IGLC2
N5PMPB155	IGHV5-51*01	IGHD2-2*02	IGHJ4*02	HRSGYCSITSCSHFDY	16	1	IGHG1						IGLV2-23*03	IGLJ1*01	CSYAGSSTNYV	0	----
N5PMPB180	IGHV4-59*01	IGHD5-5*01	IGHJ5*02	GGGYFDP	8	30	IGHG3						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N7PMPB205	IGHV4-34*01	IGHD7-27*01	IGHJ2*01	GWGYWYFDL	10	1	IGHG1						IGLV2-14*03	IGLJ2*01	SSYTSSSTVV	0	IGLC7
N7PMPB218	IGHV1-46*02	IGHD6-13*01	IGHJ6*02	DLGSWHDNYYGMDV	15	23	IGHA1						IGLV2-23*02	IGLJ2*01	CSYAPSNTVV	15	IGLC2
N7PMPB226	IGHV4-34*01	IGHD1-26*01	IGHJ3*02	ELATQRGRPAHNGFDI	17	13	IGHA1						IGLV2-23*02	IGLJ3*02	RSYAGISPLWV	9	IGLC7
N7PMPB228	IGHV3-23*01	IGHD2-21*02	IGHJ4*02	DRFRNKYEDYQYCGGDCRSPPPPDY	26	5	IGHG2						IGLV1-44*01	IGLJ3*02	AWDDSLNGWV	4	IGLC1
N7PMPB259	IGHV4-31*03	IGHD3-10*02	IGHJ2*01	APYNDGLPGFGYFDL	16	25	IGHG4						IGLV3-21*02	IGLJ2*01	QVWDSSSDRVV	0	IGLC7
N7PMPB267	IGHV3-15*07	IGHD5-12*01	IGHJ4*02	GYNQYFDN	8	8	IGHA2						IGLV2-11*03	IGLJ3*02	CSHGGTYTYWV	9	IGLC2
N7PMPB269	IGHV4-31*03	IGHD5-5*01	IGHJ3*02	DSGYNYGYVFGAFDI	15	4	IGHG2						IGLV1-44*01	IGLJ2*01	AAWDDSLNGVV	4	----

Table 18: Vax3 Ig clusters and related antibody "families"

LABEL		HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
Family	Cluster ID	Antibody Name	VH	D	JH	CDR3	CDR3 Lenght	SHM	Constant	VK	JK	CDR3	SHM	Constant	VL	JL	CDR3	SHM	Constant
		N6PMPB091	IGHV4-39*07	IGHD6-13*01	IGHJ4*01	SRVIAAASFDY	11	3	IGHG2	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	6	IGKC	IGLV1-40*01	IGLJ1*01	QSYDSLSGYV	24	IGLC1
		N6PMPB148	IGHV4-39*07	IGHD2-2*03	IGHJ4*02	SRVVAAAMFDY	11	18	IGHG1	IGKV1-33*01	IGKJ1*01	QQFDTLPGT	11	IGKC					
Convergent or Divergent Evolution	Cluster 1	N6PMPB161	IGHV4-39*07	IGHD6-6*01	IGHJ4*02	SRVIGAGSFDY	11	3	IGHG1	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	0	IGKC					
		N6PMPB223	IGHV1-69*01	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	21	IGHG1	IGKV1D-39*01	IGKJ1*01	QQGYSTPPWT	33	IGKC					
		N6PMPB321	IGHV1-69*09	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	19	IGHG1	IGKV4-1*01	IGKJ2*01	QQCYSVPYT	29	IGKC					
	Cluster 2	N6PMPB344	IGHV1-69*09	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	20	IGHG1	IGKV4-1*01	IGKJ2*01	QQHHSTPYT	8	IGKC					
		N6PMPB373	IGHV1-69*09	IGHD3-3*01	IGHJ5*02	SLKDYYDFSWFDP	13	5	IGHG2	IGKV4-1*01	IGKJ4*01	QQYYSPPLT	7	IGKC					
Convergent or Civergent Evolution		N6PMPB384	IGHV1-69*04	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	18	IGHG1	IGKV4-1*01	IGKJ4*01	QHYYSPPLG	19	IGKC					
		N6PMPB013	IGHV3-66*04	IGHD4-17*01	IGHJ6*02	SIATVTTTVDYYYYGMDV	18	6	IGHA1						IGLV3-25*02	IGLJ3*02	QSQDTSGTYGV	5	IGLC2
Convergent or Divergent Evolution		N6PMPB129	IGHV3-66*04	IGHD3-22*01	IGHJ6*02	NIYYDAGTADHYYYGMDV	18	12	IGHG1						IGLV3-25*02	IGLJ3*02	QSQDSSGTIGV	27	IGLC7
		N6PMPB283	IGHV2-26*01	IGHD5-24*01	IGHJ4*02	HSWVELWFDS	10	4	IGHA1	IGKV1-5*01	IGKJ2*02	QHYNTYSPT	24	IGKC					
		N6PMPB355	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	10	IGHG1	IGKV1-5*01	IGKJ2*02	QQYNGYSPT	13	IGKC					
		N6PMPB361	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1	IGKV1-5*01	IGKJ2*02	QHYKTYSPT	16	IGKC					
		N6PMPB369	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIELWFDY	10	6	IGHG1	IGKV1-5*01	IGKJ2*02	QHYNTYSPT	6	IGKC					
Convergent or Divergent Evolution	Cluster 3	N6PMPB377	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1	IGKV1-5*01	IGKJ2*02	QHYKTYSPT	12	IGKC					
		N6PMPB328	IGHV3-73*01	IGHD3-16*02	IGHJ4*01	VTPFGFDY	8	30	IGHG1	IGKV3-11*01	IGKJ4*01	QQRSDWPLT	16	IGKC	IGLV3-10*01	IGLJ3*01	YSTDSSDTYVV	10	IGLC2
		N6PMPB100	IGHV3-73*01	IGHD3-16*02	IGHJ4*01	VTPFGFDY	8	26	IGHG1						IGLV3-10*01	IGLJ3*01	YSTDSSDTYVV	8	IGLC2
	Cluster 4	N6PMPB274	IGHV3-73*01	IGHD3-16*02	IGHJ4*01	VTPFGFDY	8	31	IGHG1						IGLV3-10*01	IGLJ3*01	YSTDSSDTYVV	8	IGLC2
		N6PMPB114	IGHV3-23*04	IGHD3-3*02	IGHJ2*01	DPFLIRVAGDWYFDL	15	19	IGHA1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	12	IGLC2
		N6PMPB138	IGHV3-23*04	IGHD2-21*02	IGHJ2*01	DPFMMRLAGDWYFDL	15	22	IGHG1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	15	IGLC2
		N6PMPB267	IGHV3-23*04	IGHD3-9*01	IGHJ2*01	DPFMMRLAGDWYFDL	15	24	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDTSLGGYVL	11	IGLC2
		N6PMPB371	IGHV3-23*04	IGHD3-16*01	IGHJ2*01	DPFLIRLGGDYYFDL	15	36	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDSSLGGYVV	11	IGLC2
Convergent or Divergent Evolution	Cluster 5	N6PMPB008	IGHV3-23*04	IGHD1-26*01	IGHJ2*01	DPFLIRLAGDYYFDL	15	22	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDTSLGGYVL	12	IGLC2

Table 19: Vax4 Ig clusters and related antibody "families"

	HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
Cluster ID	Antibody Name	VH	D	JH	CDR3	CDR3 Lenght	SHM	Constant	Vk	Jk	CDR3	SHM	Constant	Vl	Jl	CDR3	SHM	Constant
	N7PMPB206	IGHV3-30*18	IGHD3-10*02	IGHJ5*02	DRYPGEILIWFDP	13	7	IGHA1	IGKV2D-26*01	IGKJ3*01	MQDSQDPLFT	10	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	8	
	N7PMPB241	IGHV3-30*18	IGHD2-2*02	IGHJ5*02	DRYPGEILIWFDP	13	6	IGHA1	IGKV2D-26*01	IGKJ3*01	MQDSQDPLFT	8	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	7	
Cluster 1																		
	N7PMPB236	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	16	IGKC					
Cluster 2	N7PMPB262	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	13	IGKC					

NB: Ig "families" includes groups of Igs that originate from a common Ig progenitor or converge from different Ig progenitors and share similarities in Ig gene features. Cluster antibodies originate from a common Ig progenitor sharing the same heavy chain, light chain, isotypes, CDR3 length and same or similar CDR3 composition and numbers of somatic hypermutations.

Table 20: Vax2 monoclonal antibodies reactive to Bexsero in ELISA

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N5PMPB045	IGHV3-7*01	IGHD6-25*01	IGHJ4*02	TYRSSGTDY	9	14	IGHG2	IGKV2-30*01	IGKJ3*01	VQLTQWPFT	7	IGKC					
N5PMPB112	IGHV3-23*04	IGHD4-17*01	IGHJ3*02	KLHDYVQGGAFDI	13	25	IGHG1	IGKV1-5*03	IGKJ2*01	QQYYSYLYT	14	IGKC					
N5PMPB029	IGHV1-69*01	IGHD4-17*01	IGHJ5*02	GGWTISTESTRGHLEQ	17	31	IGHA1						IGLV8-61*01	IGLJ3*02	VLYLGSYNWV	13	
N5PMPB085	IGHV3-30*14	IGHD3-10*02	IGHJ5*02	DKGNYYTSGSHFRVLDS	17	33	IGHG1	IGKV3-20*01	IGKJ5*01	QQYYDSPPIT	30	IGKC					
N5PMPB023	IGHV3-53*01	IGHD2-15*01	IGHJ4*02	FCSAGTCDSEEAFDY	16	22	IGHA1						IGLV2-23*02	IGLJ1*01	FSYARGDSYV	21	
N5PMPB134	IGHV4-34*01	IGHD3-10*01	IGHJ4*02	GRGKLQWFAELYH	13	4	IGHG1	IGKV3-20*01	IGKJ2*01	QQYGSSPRT	5	IGKC					
N5PMPB164	IGHV4-39*01	IGHD4-17*01	IGHJ2*01	HATVTAGGDFDL	13	10	IGHA1						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB111	IGHV3-7*01	IGHD6-19*01	IGHJ5*02	RMVAVIGTGTFA	12	17	IGHG1	IGKV4-1*01	IGKJ1*01	LQNYNGRWT	23	IGKC					
N5PMPB062	IGHV1-46*01	IGHD5-24*01	IGHJ5*02	WLKGDETDH	9	13	IGHA1	IGKV1-17*01	IGKJ1*01	LQHDTYPWT	13	IGKC					
N5PMPB074	IGHV1-69*06	IGHD3-16*02	IGHJ5*02	DQVPWLGAEVPVALES	16	45	IGHG3	IGKV3-20*01	IGKJ4*01	HQYGDSPLT	25	IGKC					
N5PMPB045	IGHV3-7*01	IGHD6-25*01	IGHJ4*02	TYRSSGTDY	9	14	IGHG2	IGKV2-30*01	IGKJ3*01	VQLTQWPFT	7	IGKC					
N5PMPB131	IGHV3-30*18	IGHD6-19*01	IGHJ4*02	GNAMGPHGCFDY	12	11	IGHG1	IGKV1-39*01	IGKJ4*01	QQSFRTPLT	14	IGKC					
N5PMPB013	IGHV4-34*01	IGHD3-9*01	IGHJ6*03	GRRYFDWPTFVMDV	14	1	IGHM	IGKV1-17*01	IGKJ4*01	LQHNSYPLT	6	IGKC					
N5PMPB118	IGHV3-9*01	IGHD3-16*02	IGHJ2*01	NGGGSTWHWYFDL	13	16	IGHG1	IGKV3D-15*01	IGKJ3*01	QQYNNWPLS	5	IGKC					
N5PMPB156	IGHV1-46*02	IGHD1-26*01	IGHJ5*02	DYSHTNWLGP	10	30	IGHA1	IGKV3-20*01	IGKJ2*02	QHYDHSPRVYT	23	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB136	IGHV3-23*04	IGHD6-19*01	IGHJ5*01	LQSSGWYSDL	10	19	IGHG1	IGKV1-39*01	IGKJ2*01	QQSYNTYT	10	IGKC					
N5PMPB127	IGHV1-46*02	IGHD3-10*01	IGHJ6*02	DLTMVRGLVGYYGMDV	16	24	IGHG1	IGKV2-28*01	IGKJ2*01	MQALQTPPYT	6	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB037	IGHV3-7*01	IGHD5-24*01	IGHJ4*02	AKHGY	5	0	IGHG1	IGKV3-11*01	IGKJ4*02	QQRSNWPWT	3	IGKC					
N5PMPB153	IGHV3-43*01	IGHD3-3*01	IGHJ6*02	GGYYDFWSGYDKRPYHYYGMDV	23	5	IGHA1						IGLV2-14*01	IGLJ1*01	SSYTGSSTDV	2	IGLC1
N5PMPB014	IGHV1-2*02	IGHD2-2*02	IGHJ4*01	GYSDIIGRCLDY	12	15	IGHA2	IGKV1-9*01	IGKJ1*01	QQLNSYPRT	4	IGKC					
N5PMPB012	IGHV3-9*01	IGHD5-5*01	IGHJ4*02	DIHPLKIQLWLGGFDY	16	7	IGHG1						IGLV2-23*03	IGLJ1*01	CSYAGSSTYV	3	
N5PMPB025	IGHV3-21*01	IGHD1-14*01	IGHJ6*03	VANLRANRYYYYYMDV	16	0	IGHM	IGKV3-11*01	IGKJ2*01	QQRSNWLYT	3	IGKC					
N5PMPB056	IGHV4-4*07	IGHD2-2*02	IGHJ5*02	GGYFSTSGSNWFDP	15	7	IGHM						IGLV2-8*02	IGLJ1*01	SSYAGSNIYV	1	
N5PMPB081	IGHV3-48*02	IGHD6-19*01	IGHJ4*02	SGWSRYHFDH	10	10	IGHG1	IGKV3-20*01	IGKJ1*01	QQYGSSLWT	6	IGKC					
N5PMPB003	IGHV3-23*04	IGHD3-22*01	IGHJ4*02	DRYSTASYYFDY	12	31	IGHA1	IGKV1-12*01	IGKJ2*02	QLPNSLPPIYA	24	IGKC					
N5PMPB088	IGHV3-9*01	IGHD2-2*02	IGHJ1*01	ESPPCTNTWSSFHR	14	22	IGHG2	IGKV3-20*01	IGKJ4*02	HQYGSSPLT	13	IGKC					

Table 21: Vax4 monoclonal antibodies reactive to Bexsero in ELISA

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N7PMPB261	IGHV3-23*01	IGHD1-1*01	IGHJ6*02	EYRLLTTATHSMDV	14	12	IGHA2						IGLV2-14*01	IGLJ1*01	FSYTITSTPYV	8	IGLC1
N7PMPB228	IGHV3-23*01	IGHD2-21*02	IGHJ4*02	DRFRNKYEDYQYCGGDCRSPPPPDY	26	5	IGHG2						IGLV1-44*01	IGLJ3*02	AWDDSLNGWV	4	IGLC1
N7PMPB259	IGHV4-31*03	IGHD3-10*02	IGHJ2*01	APYNDGLPGFGYFDL	16	25	IGHG4						IGLV3-21*02	IGLJ2*01	QVWDSSSDRVV	0	IGLC7
N7PMPB258	IGHV4-59*01	IGHD3-9*01	IGHJ4*02	GTPSYDILTGYETAYFDY	18	0	IGHM	IGKV3-20*01	IGKJ5*01	QQYGSSPIT	4	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N7PMPB241	IGHV3-30*18	IGHD2-2*02	IGHJ5*02	DRYPGEILIWFDP	13	6	IGHA1	GKV2D-26*0	IGKJ3*01	MQDSQDPLFT	8	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	7	IGLC1
N7PMPB276	IGHV4-39*01	IGHD6-19*01	IGHJ4*02	RLAVGWPRDYFDY	13	24	IGHG1	IGKV1-12*01	IGKJ1*01	QQAHSFPWT	12	IGKC					
N7PMPB206	IGHV3-30*18	IGHD3-10*02	IGHJ5*02	DRYPGEILIWFDP	13	7	IGHA1	GKV2D-26*0	IGKJ3*01	MQDSQDPLFT	10	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	8	IGLC1
N7PMPB236	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	18	IGKC					
N7PMPB262	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	13	IGKC					
N7PMPB245	IGHV3-74*01	IGHD6-19*01	IGHJ4*02	DTRSGWYGGFDY	12	0	IGHM	IGKV4-1*01	IGKJ2*01	QQYYSTPYT	1	IGKC					
N5PMPB182	IGHV1-8*01	IGHD3-16*02	IGHJ5*02	GAGEGGRDWFDP	12	20	IGHA2	IGKV4-1*01	IGKJ4*01	QQYHSKPPT	11	IGKC					
N7PMPB196	IGHV1-18*01	IGHD6-13*01	IGHJ6*02	EGGVPPEGQQLHILRGYYYYGMDV	25	1	IGHG1	IGLV1-36*01	IGLJ3*02	AAWDDSLNGP	2	IGLC7					
N7PMPB255	IGHV3-74*03	IGHD3-10*02	IGHJ3*02	DRGWNALDL	9	14	IGHA2	IGKV2-29*02	IGKJ3*01	MQGLHLPLT	11	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N7PMPB281	IGHV5-51*01	IGHD6-13*01	IGHJ3*02	VRVAAAGRGMYVGNAFDI	18	0	IGHM	IGKV3-20*01	IGKJ3*01	QQYGSSPLFT	4	IGKC					
N7PMPB242	IGHV5-10-1*01	IGHD1-7*01	IGHJ4*03	VPWYNSSTWFAPNW	14	22	IGHA2	IGKV1-16*02	IGKJ4*01	QQYKSYPET	11	IGKC					
N7PMPB235	IGHV3-64*01	IGHD2-15*01	IGHJ4*02	GGYCSGGSCYLPY	13	0	IGHG1	IGKV4-1*01	IGKJ1*01	QQYYSTPQT	1	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1

Table 22: Vax3 monoclonal antibodies reactive to Bexsero in ELISA

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N6PMPB349	IGHV5-51*01	IGHD3-3*02	IGHJ5*02	RAYGGSELATNNWFDP	16	36	IGHG2	IGKV2-30*01	IGKJ1*01	MQRSRRPWA	32	IGKC					
N6PMPB015	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DQNPLLWFGGSLDY	14	10	IGHG4						IGLV1-44*01	IGLJ3*01	AAWDDSLNAHR	1	IGLC7
N6PMPB177	IGHV3-9*01	IGHD3-10*01	IGHJ6*02	DRVTMVRGYGMDV	13	2	IGHG2	IGKV4-1*01	IGKJ2*01	QQHHSTPYT	12	IGKC					
N6PMPB134	IGHV4-59*01	IGHD3-22*01	IGHJ5*02	GPTMIQE	7	1	IGHG3	IGKV1-5*01	IGKJ2*01	QHYKTYSPT	12	IGKC					
N6PMPB198	IGHV4-39*01	IGHD3-3*02	IGHJ4*02	RPFSALLEWLFTPFDN	16	17	IGHG4	IGKV1-5*03	IGKJ3*01	QQYNSYLFT	14	IGKC					
N6PMPB147L	IGHV4-4*07	IGHD3-10*01	IGHJ6*02	AGAVLTPLWFGETHYYYYPMDV	22	13	IGHG1						IGLV3-21*01	IGLJ3*02	QVWHTSSDHRGV	26	IGLC3
N6PMPB105	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	SGAWWAFDY	9	22							IGLV2-8*01	IGLJ3*02	SSYGGSANVL	10	IGLC7
N6PMPB279	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DSYGSGIYRRMDN	13	35	IGHA1						IGLV2-11*01	IGLJ1*01	CSFAGKFTYV	24	IGLC1
N6PMPB303	IGHV1-8*01	IGHD3-10*01	IGHJ6*02	AWDVEDREDFGMDV	14	23	IGHA1	IGKV1D-39*01	IGKJ1*01	QQSSTTPWT	18	IGKC					
N6PMPB113	IGHV3-11*01	IGHD3-22*01	IGHJ6*02	NRGGFYIYGMDV	12	17	IGHG1						IGLV6-57*01	IGLJ3*01	QSYDSSDVV	9	
N6PMPB181	IGHV4-39*07	IGHD3-10*02	IGHJ4*02	GTSFHYGSGSFYKQPPFDS	19	33	IGHG1	IGKV4-1*01	IGKJ4*01	QQYYNTPLT	9	IGKC					
N6PMPB174	IGHV3-23*04	IGHD2-21*01	IGHJ6*04	DRGKERRPKQTGKSYYYYGLDV	23	2	IGHA1	IGKV3-15*01	IGKJ2*02	QQYNNWPLYT	6	IGKC					
N6PMPB147k	IGHV4-4*07	IGHD3-10*01	IGHJ6*02	AGAVLTPLWFGETHYYYYPMDV	22	13	IGHG1	IGKV3-20*01	IGKJ2*01	QQYGTSHLYT	12	IGKC					
N6PMPB344	IGHV1-69*09	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	20	IGHG1	IGKV4-1*01	IGKJ2*01	QHHYRIPYT	27	IGKC					
N6PMPB247	IGHV4-59*01	IGHD1-26*01	IGHJ4*02	GGGWDLLDY	9	19	IGHG2	IGKV1D-39*01	IGKJ3*01	QQSGTTPL	13	IGKC					
N6PMPB179	IGHV3-7*01	IGHD3-3*01	IGHJ4*02	APQYFDFWSSDY	12	2	IGHG1						IGLV3-1*01	IGLJ3*01	QAWDSSTVI	2	IGLC7
N6PMPB321	IGHV1-69*09	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	19	IGHG1	IGKV4-1*01	IGKJ2*01	QQCYSVPYT	29	IGKC					
N6PMPB302	IGHV3-7*01	IGHD3-9*01	IGHJ4*02	GGQAWIQLWYFDY	13	10	IGHG1	IGKV1-6*01	IGKJ3*01	LQDYNYPFT	10	IGKC					
N6PMPB112	IGHV3-15*01	IGHD6-25*01	IGHJ6*02	GVGMGGMDV	9	6	IGHG1						IGLV9-49*01	IGLJ3*02	GADHGSGSNFFWV	2	IGLC7
N6PMPB124	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DRLKYYGSGSPLDY	14	8	IGHG1						IGLV3-21*01	IGLJ3*02	QVWHTSSDHRGV	8	IGLC2
N6PMPB344	IGHV1-69*09	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	20	IGHG1	IGKV4-1*01	IGKJ2*01	QHHYRIPYT	27	IGKC					
N6PMPB325	IGHV3-72*01	IGHD2-21*02	IGHJ4*02	LSGSY	5	15	IGHM						IGLV1-47*01	IGLJ3*02	AVWDDSLSGRV		IGLC2
N6PMPB298	IGHV3-11*01	IGHD3-3*01	IGHJ6*02	DQAALKNFDFWSAYPAPQYYFGMAV	25	26	IGHG4	IGKV3-15*01	IGKJ2*01	QHYNNWPYT	10	IGKC					
N6PMPB297	IGHV1-3*01	IGHD2-2*02	IGHJ4*02	GYCSSTSCQYYFDY	14	20		IGKV4-1*01	IGKJ2*01	QQYYSIPYT	13	IGKC					
N6PMPB100	IGHV3-73*01	IGHD3-16*02	IGHJ4*01	VTPFGFDY	8	26	IGHG1						IGLV3-10*01	IGLJ3*01	YSTDSSDTYVV	8	IGLC2
N6PMPB250	IGHV3-11*01	IGHD3-16*02	IGHJ5*02	GRGSYLPPGLDH	12	22	IGHA2						IGLV3-21*02	IGLJ3*01	QVWDSNRQHPYMI	20	IGLC7
N6PMPB356	IGHV3-11*01	IGHD4-17*01	IGHJ6*02	GTYGDYRQVGMDV	13	23	IGHA2						IGLV1-47*01	IGLJ3*01	ATWDDSLGGL	16	
N6PMPB153	IGHV3-15*01	IGHD5-5*01	IGHJ4*02	VFLNDRLLGYTYGWSSVFDY	20	14	IGHG1	IGKV1D-39*01	IGKJ3*01	QQSFSSLGPT	24	IGKC					
N6PMPB365	IGHV3-23*04	IGHD5-24*01	IGHJ4*01	ALYNDRWFFDY	11	31	IGHA1	IGKV3D-11*01	IGKJ4*01	QQRGNWPLT	13	IGKC					
N6PMPB294	IGHV1-8*01	IGHD7-27*01	IGHJ1*01	AVHTNYMGQVLYSLDS	16	18		IGKV1-39*01	IGKJ4*01	QQSYLTPFT	18	IGKC					
N6PMPB091_K	IGHV4-39*07	IGHD6-13*01	IGHJ4*01	SRVIAAASFDY	11	3	IGHG2	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	6	IGKC					
N6PMPB091_L	IGHV4-39*07	IGHD6-13*01	IGHJ4*01	SRVIAAASFDY	11	3	IGHG2						IGLV1-40*01	IGLJ1*01	QSYDSLSGYV	24	IGLC1
N6PMPB148	IGHV4-39*07	IGHD2-2*03	IGHJ4*02	SRVVAAAMFDY	11	18	IGHG1	IGKV1-33*01	IGKJ1*01	QQFDTLPGT	11	IGKC					
N6PMPB161	IGHV4-39*07	IGHD6-6*01	IGHJ4*02	SRVIGAGSFDY	11	3	IGHG1	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	0	IGKC					
N6PMPB138	IGHV3-23*04	IGHD2-21*02	IGHJ2*01	DPFMMRLAGDWYFDL	15	22	IGHG1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	15	IGLC2
N6PMPB267	IGHV3-23*04	IGHD3-9*01	IGHJ2*01	DPFMMRLAGDWYFDL	15	24	IGHG1						IGLV1-40*02	IGLJ3*01	QSTYDPSRGGYVV	39	
N6PMPB114	IGHV3-23*04	IGHD3-3*02	IGHJ2*01	DPFLIRVAGDWYFDL	15	19	IGHA1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	12	
N6PMPB008	IGHV3-23*04	IGHD1-26*01	IGHJ2*01	DPFLIRLAGDYYFDL	15	22	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDTSLGGYVL	12	IGLC2
N6PMPB371	IGHV3-23*04	IGHD3-16*01	IGHJ2*01	DPFLIRLGGDYYFDL	15	36	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDSSLGGYVV	11	
N6PMPB355	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	10	IGHG1	IGKV1-5*01	IGKJ2*02	QQYNGYSPT	13	IGKC					
N6PMPB361	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1	IGKV1-5*01	IGKJ2*02	QHYKTYSPT	16	IGKC					
N6PMPB369	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIELWFDY	10	6	IGHG1	IGKV1-5*01	IGKJ2*02	QHYNTYSPT	6	IGKC					
N6PMPB377	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1	IGKV1-5*01	IGKJ2*02	QHYKTYSPT	12	IGKC					
N6PMPB384	IGHV1-69*04	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	18	IGHG1	IGKV4-1*01	IGKJ4*01	QHYYSPPLG	19	IGKC					
N6PMPB373	IGHV1-69*09	IGHD3-3*01	IGHJ5*02	SLKDYYDFSWFDP	13	5	IGHG2	IGKV4-1*01	IGKJ4*01	QQYYSPPLT	7	IGKC					
N6PMPB135	IGHV3-15*01	IGHD2-15*01	IGHJ4*02	DCTAGDSCSPYFDS	14	22	IGHA1	IGKV1-16*02	IGKJ5*01	QQYSAYPLT	25	IGKC					
N6PMPB224	IGHV4-59*08	IGHD2-2*02	IGHJ3*02	RWEDIVVVPAALAVGSVSYADAFDI	25	1	IGHG1	IGKV1D-39*01	IGKJ5*01	QQSYTTPIT	5	IGKC					
N6PMPB346	IGHV1-46*01	IGHD3-16*02	IGHJ4*02	DSHDYVWGRFDY	12	11	IGHG1	IGLV1-40*02	IGLJ3*01	QSYDSSLSVV	3	IGLC2					
N6PMPB214	IGHV3-11*01	IGHD5-5*01	IGHJ5*02	GHSYGSF	7	19	IGHM	IGKV3-20*01	IGKJ5*01	QRYGSSLGT	18	IGKC					
N6PMPB190	IGHV3-33*01	IGHD3-22*01	IGHJ4*02	RGGGYYDSSGYDGSFDY	17	4	IGHG2						IGLV3-25*02	IGLJ3*02	QSADSSGSWV	5	IGLC7
N6PMPB318	IGHV3-23*04	IGHD2-21*02	IGHJ4*02	VPQVTAIHLVDS	13	34	IGHA1	IGKV1-5*03	IGKJ4*02	QRYDSYSGT	26	IGKC					
N6PMPB345	IGHV4-34*02	IGHD3-9*01	IGHJ6*02	RRNWYYGMDA	10	34	IGHG3	IGKV1-5*03	IGKJ2*01	QQYNRYSFHT	18	IGKC					
N6PMPB350	IGHV3-21*01	IGHD6-13*01	IGHJ4*02	AAAGIYYFDY	10	16	IGHG2	IGKV3-20*01	IGKJ2*01	QQYGSSSRYT	13	IGKC					
N6PMPB033	IGHV7-4-1*02	IGHD3-22*01	IGHJ6*02	IYHHDSGGPDYHGMDV	16	19	IGHA1						IGLV3-25*02	IGLJ3*02	QSADSSGTYWV	19	IGLC7
N6PMPB258	IGHV3-30*04	IGHD3-22*01	IGHJ4*02	DREWLLLHY	9	2	IGHG1	IGKV1-16*02	IGKJ5*01	QQYNSYPIT	4	IGKC					
N6PMPB013	IGHV3-66*04	IGHD4-17*01	IGHJ6*02	SIATVTTTVDYYYYGMDV	18	6	IGHA1						IGLV3-25*02	IGLJ3*02	QSQDTSGTYGV	5	IGLC2
N6PMPB171	IGHV3-9*01	IGHD3-10*01	IGHJ3*02	DTTPLVWFGELLGDDAFDI	19	3	IGHG1						IGLV1-41*01	IGLJ3*02	AAWDDSLNGWV	1	IGLC7

Table 23: Vax3 Monoclonal antibodies reactive to Bexsero in both ELISA and Western Blot

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N6PMPB091_K	IGHV4-39*07	IGHD6-13*01	IGHJ4*01	SRVIAAASFDY	11	3	IGHG2	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	6	IGKC					
N6PMPB091_L	IGHV4-39*07	IGHD6-13*01	IGHJ4*01	SRVIAAASFDY	11	3	IGHG2						IGLV1-40*01	IGLJ1*01	QSYDSLSGYV	24	IGLC1
N6PMPB148	IGHV4-39*07	IGHD2-2*03	IGHJ4*02	SRVVAAAMFDY	11	18	IGHG1	IGKV1-33*01	IGKJ1*01	QQFDTLPGT	11	IGKC					
N6PMPB161	IGHV4-39*07	IGHD6-6*01	IGHJ4*02	SRVIGAGSFDY	11	3	IGHG1	IGKV1-33*01	IGKJ1*01	QQYDNLPPA	0	IGKC					
N6PMPB138	IGHV3-23*04	IGHD2-21*02	IGHJ2*01	DPFMMRLAGDWYFDL	15	22	IGHG1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	15	IGLC2
N6PMPB267	IGHV3-23*04	IGHD3-9*01	IGHJ2*01	DPFMMRLAGDWYFDL	15	24	IGHG1						IGLV1-40*02	IGLJ3*01	QSTYDPSRGGYVV	39	
N6PMPB114	IGHV3-23*04	IGHD3-3*02	IGHJ2*01	DPFLIRVAGDWYFDL	15	19	IGHA1						IGLV1-40*01	IGLJ3*01	QSYDTSLGGYVV	12	
N6PMPB008	IGHV3-23*04	IGHD1-26*01	IGHJ2*01	DPFLIRLAGDYYFDL	15	22	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDTSLGGYVL	12	IGLC2
N6PMPB371	IGHV3-23*04	IGHD3-16*01	IGHJ2*01	DPFLIRLGGDYYFDL	15	36	IGHG1						IGLV1-40*02	IGLJ3*01	QSYDSSLGGYVV	11	
N6PMPB355	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	10	IGHG1	IGKV1-5*01	IGKJ2*02	QQYNGYSPT	13	IGKC					
N6PMPB361	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1	IGKV1-5*01	IGKJ2*02	QHYKTYSPT	16	IGKC					
N6PMPB369	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIELWFDY	10	6	IGHG1	IGKV1-5*01	IGKJ2*02	QHYNTYSPT	6	IGKC					
N6PMPB377	IGHV2-26*01	IGHD5-5*01	IGHJ4*02	HSWIQLWFDY	10	8	IGHG1	IGKV1-5*01	IGKJ2*02	QHYKTYSPT	12	IGKC					
N6PMPB384	IGHV1-69*04	IGHD5-12*01	IGHJ5*02	GDDSGFDLSWFDS	13	18	IGHG1	IGKV4-1*01	IGKJ4*01	QHYYSPPLG	19	IGKC					
N6PMPB373	IGHV1-69*09	IGHD3-3*01	IGHJ5*02	SLKDYYDFSWFDP	13	5	IGHG2	IGKV4-1*01	IGKJ4*01	QQYYSPPLT	7	IGKC					
N6PMPB135	IGHV3-15*01	IGHD2-15*01	IGHJ4*02	DCTAGDSCSPYFDS	14	22	IGHA1	IGKV1-16*02	IGKJ5*01	QQYSAYPLT	25	IGKC					
N6PMPB224	IGHV4-59*08	IGHD2-2*02	IGHJ3*02	RWEDIVVVPAALAVGSVSYADAFDI	25	1	IGHG1	IGKV1D-39*01	IGKJ5*01	QQSYTTPIT	5	IGKC					
N6PMPB346	IGHV1-46*01	IGHD3-16*02	IGHJ4*02	DSHDYVWGRFDY	12	11	IGHG1	IGLV1-40*02	IGLJ3*01	QSYDSSLSVV	3	IGLC2					
N6PMPB214	IGHV3-11*01	IGHD5-5*01	IGHJ5*02	GHSYGSF	7	19	IGHM	IGKV3-20*01	IGKJ5*01	QRYGSSLGT	18	IGKC					
N6PMPB190	IGHV3-33*01	IGHD3-22*01	IGHJ4*02	RGGGYYDSSGYDGSFDY	17	4	IGHG2						IGLV3-25*02	IGLJ3*02	QSADSSGSWV	5	IGLC7
N6PMPB318	IGHV3-23*04	IGHD2-21*02	IGHJ4*02	VPQVTAIHLVDS	13	34	IGHA1	IGKV1-5*03	IGKJ4*02	QRYDSYSGT	26	IGKC					
N6PMPB345	IGHV4-34*02	IGHD3-9*01	IGHJ6*02	RRNWYYGMDA	10	34	IGHG3	IGKV1-5*03	IGKJ2*01	QQYNRYSFHT	18	IGKC					
N6PMPB350	IGHV3-21*01	IGHD6-13*01	IGHJ4*02	AAAGIYYFDY	10	16	IGHG2	IGKV3-20*01	IGKJ2*01	QQYGSSSRYT	13	IGKC					
N6PMPB033	IGHV7-4-1*02	IGHD3-22*01	IGHJ6*02	IYHHDSGGPDYHGMDV	16	19	IGHA1						IGLV3-25*02	IGLJ3*02	QSADSSGTYWV	19	IGLC7
N6PMPB258	IGHV3-30*04	IGHD3-22*01	IGHJ4*02	DREWLLLHY	9	2	IGHG1	IGKV1-16*02	IGKJ5*01	QQYNSYPIT	4	IGKC					
N6PMPB013	IGHV3-66*04	IGHD4-17*01	IGHJ6*02	SIATVTTTVDYYYYGMDV	18	6	IGHA1						IGLV3-25*02	IGLJ3*02	QSQDTSGTYGV	5	IGLC2
N6PMPB171	IGHV3-9*01	IGHD3-10*01	IGHJ3*02	DTTPLVWFGELLGDDAFDI	19	3	IGHG1						IGLV1-41*01	IGLJ3*02	AAWDDSLNGWV	1	IGLC7

Table 24: Vax2 monoclonal antibody reactivity data

ANTIBODY	EXPRESSED	Bexsero (AUC+-SEM)	Insulin (AUC+-SEM)	LPS (AUC+-SEM)	dsDNA (AUC+-SEM)	fHbp	NadA	NHBA	PorA P1.4
N5PMPB110K	FALSE	-	-	-	-	-	-	-	-
N5PMPB116K	TRUE	1.30 ± 0	2.55 ± 0.00	1.15 ± 0.00	1.31 ± 0.05	FALSE	FALSE	FALSE	FALSE
N5PMPB127K	TRUE	6.05 ± 1.87	5.25 ± 0.29	1.96 ± 0.28	1.19 ± 0.05	FALSE	FALSE	FALSE	FALSE
N5PMPB156K	TRUE	1.59 ± 0.45	2.28 ± 0.04	2.04 ± 0.82	0.84 ± 0.06	FALSE	FALSE	FALSE	FALSE
N5PMPB165K	FALSE	-	-	-	-	-	-	-	-
N5PMPB166K	TRUE	0.53 ± 0.06	1.33 ± 0.28	0.57 ± 0.04	0.60 ± 0.05	FALSE	FALSE	FALSE	FALSE
N5PMPB003	TRUE	3.78 ± 1.04	2.40 ± 0.53	1.10 ± 0.28	1.01 ± 0.00	FALSE	FALSE	FALSE	FALSE
N5PMPB006	FALSE	-	-	-	-	-	-	-	-
N5PMPB013	TRUE	1.91 ± 0.47	2.41 ± 0.17	2.27 ± 0.77	1.20 ± 0.15	FALSE	FALSE	FALSE	FALSE
N5PMPB014	TRUE	2.29 ± 0.00	2.00 ± 0.00	0.86 ± 0.00	0.90 ± 0.00	FALSE	FALSE	FALSE	FALSE
N5PMPB025	TRUE	4.20 ± 0.95	3.92 ± 0.30	1.32 ± 0.19	1.00 ± 0.07	FALSE	FALSE	FALSE	FALSE
N5PMPB027	FALSE	-	-	-	-	-	-	-	-
N5PMPB033	TRUE	0.48 ± 0.07	0.96 ± 0.23	0.48 ± 0.04	0.50 ± 0.04	FALSE	FALSE	FALSE	FALSE
N5PMPB037	TRUE	1.40 ± 0.10	2.40 ± 0.48	1.13 ± 0.23	1.22 ± 0.12	FALSE	FALSE	FALSE	FALSE
N5PMPB038	FALSE	-	-	-	-	-	-	-	-
N5PMPB045	TRUE	2.11 ± 0.57	2.32 ± 0.13	1.21 ± 0.36	1.04 ± 0.10	FALSE	FALSE	FALSE	FALSE
N5PMPB049	TRUE	0.60 ± 0.09	1.46 ± 0.40	0.55 ± 0.04	0.58 ± 0.06	FALSE	FALSE	FALSE	FALSE
N5PMPB052	TRUE	0.54 ± 0.09	1.07 ± 0.30	0.50 ± 0.02	0.52 ± 0.06	FALSE	FALSE	FALSE	FALSE
N5PMPB060	FALSE	-	-	-	-	-	-	-	-
N5PMPB062	TRUE	2.62 ± 0.00	2.71 ± 0.00	0.86 ± 0.00	0.98 ± 0.00	FALSE	FALSE	FALSE	FALSE
N5PMPB073	FALSE	-	-	-	-	-	-	-	-
N5PMPB074	TRUE	3.91 ± 1.50	2.08 ± 0.01	1.13 ± 0.23	1.01 ± 0.08	FALSE	FALSE	FALSE	FALSE
N5PMPB078	TRUE	0.50 ± 0.06	1.13 ± 0.31	0.49 ± 0.03	0.57 ± 0.06	FALSE	FALSE	FALSE	FALSE
N5PMPB081	TRUE	3.65 ± 1.10	2.01 ± 0.31	0.83 ± 0.15	0.79 ± 0.05	FALSE	FALSE	FALSE	FALSE
N5PMPB082	FALSE	-	-	-	-	-	-	-	-
N5PMPB085	TRUE	1.35 ± 0.00	4.54 ± 0.00	1.85 ± 0.0	1.05 ± 0.00	FALSE	FALSE	FALSE	FALSE
N5PMPB088	TRUE	2.98 ± 1.39	5.06 ± 0.71	1.54 ± 0.32	1.39 ± 0.13	FALSE	FALSE	FALSE	FALSE
N5PMPB090	FALSE	-	-	-	-	-	-	-	-
N5PMPB099	FALSE	-	-	-	-	-	-	-	-
N5PMPB104	FALSE	-	-	-	-	-	-	-	-
N5PMPB112	TRUE	4.19 ± 2.13	2.39 ± 0.28	0.91 ± 0.16	0.81 ± 0.01	FALSE	FALSE	FALSE	FALSE
N5PMPB118	TRUE	4.80 ± 2.14	4.37 ± 0.14	1.53 ± 0.30	1.08 ± 0.13	FALSE	FALSE	FALSE	FALSE
N5PMPB123	TRUE	2.04 ± 0.79	2.55 ± 0.14	1.27 ± 0.23	0.96 ± 0.06	FALSE	FALSE	FALSE	FALSE
N5PMPB128	FALSE	-	-	-	-	-	-	-	-

Table 24(Continued)..

ANTIBODY	EXPRESSED	Bexsero (AUC+-SEM)	Insulin (AUC+-SEM)	LPS (AUC+-SEM)	dsDNA (AUC+-SEM)	fHbp	NadA	NHBA	PorA P1.4
N5PMPB131	TRUE	4.55 ± 1.93	3.38 ± 0.08	1.24 ± 0.18	1.00 ± 0.02	FALSE	FALSE	FALSE	FALSE
N5PMPB134	TRUE	5.65 ± 0.21	4.49 ± 0.66	1.26 ± 0.40	0.98 ± 0.12	FALSE	FALSE	FALSE	FALSE
N5PMPB135	TRUE	0.82 ± 0.13	2.26 ± 0.44	0.77 ± 0.15	0.81 ± 0.10	FALSE	FALSE	FALSE	FALSE
N5PMPB136	TRUE	3.81 ± 2.02	1.35 ± 0.03	0.89 ± 0.23	0.75 ± 0.01	FALSE	FALSE	FALSE	FALSE
N5PMPB152	FALSE	-	-	-	-	-	-	-	-
N5PMPB172	FALSE	-	-	-	-	-	-	-	-
N5PMPB182	TRUE	0.64 ± 0.10	0.64 ± 0.10	0.90 ± 0.35	0.60 ± 0.08	FALSE	FALSE	FALSE	FALSE
N5PMPB008	FALSE	-	-	-	-	-	-	-	-
N5PMPB107	FALSE	-	-	-	-	-	-	-	-
N5PMPB111	TRUE	2.31 ± 0.42	3.22 ± 0.56	1.15 ± 0.26	1.02 ± 0.03	FALSE	FALSE	FALSE	FALSE
N5PMPB141	FALSE	-	-	-	-	-	-	-	-
N5PMPB154	FALSE	-	-	-	-	-	-	-	-
N5PMPB012	TRUE	5.15 ± 0.75	2.81 ± 0.45	1.77 ± 0.84	0.76 ± 0.11	FALSE	FALSE	FALSE	FALSE
N5PMPB029	TRUE	1.19 ± 0.59	2.21 ± 0.50	1.76 ± 1.01	0.74 ± 0.07	FALSE	FALSE	FALSE	FALSE
N5PMPB053	FALSE	-	-	-	-	-	-	-	-
N5PMPB054	TRUE	0.99 ± 0.38	1.44 ± 0.15	2.27 ± 1.56	0.64 ± 0.01	FALSE	FALSE	FALSE	FALSE
N5PMPB056	TRUE	1.14 ± 0.18	3.21 ± 0.52	1.18 ± 0.33	0.84 ± 0.11	FALSE	FALSE	FALSE	FALSE
N5PMPB061	TRUE	6.10 ± 0.00	13.39 ± 0.00	2.99 ± 0.00	2.66 ± 0.00	FALSE	FALSE	FALSE	FALSE
N5PMPB063	FALSE	-	-	-	-	-	-	-	-
N5PMPB095	FALSE	-	-	-	-	-	-	-	-
N5PMPB103	TRUE	0.43 ± 0.03	0.99 ± 0.20	0.48 ± 0.06	0.47 ± 0.04	FALSE	FALSE	FALSE	FALSE
N5PMPB133	TRUE	0.53 ± 0.05	1.19 ± 0.24	0.54 ± 0.07	0.51 ± 0.05	FALSE	FALSE	FALSE	FALSE
N5PMPB142	TRUE	0.46 ± 0.04	1.16 ± 0.39	0.50 ± 0.04	0.52 ± 0.03	FALSE	FALSE	FALSE	FALSE
N5PMPB153	TRUE	2.54 ± 1.10	3.63 ± 0.51	1.18 ± 0.20	0.94 ± 0.08	FALSE	FALSE	FALSE	FALSE
N5PMPB157	FALSE	-	-	-	-	-	-	-	-
N5PMPB164	TRUE	0.84 ± 0.09	2.01 ± 0.15	0.84 ± 0.15	0.66 ± 0.07	FALSE	FALSE	FALSE	FALSE
N5PMPB015	FALSE	-	-	-	-	-	-	-	-
N5PMPB017	TRUE	2.35 ± 0.28	7.23 ± 0.44	1.75 ± 0.28	2.96 ± 0.18	FALSE	FALSE	FALSE	FALSE
N5PMPB023	TRUE	1.35 ± 0.01	1.36 ± 0.25	0.70 ± 0.12	0.86 ± 0.03	FALSE	FALSE	FALSE	FALSE
N5PMPB065	TRUE	0.67 ± 0.22	1.01 ± 0.11	0.70 ± 0.21	0.51 ± 0.06	FALSE	FALSE	FALSE	FALSE
N5PMPB114	FALSE	-	-	-	-	-	-	-	-
N5PMPB146	FALSE	-	-	-	-	-	-	-	-
N5PMPB155	FALSE	-	-	-	-	-	-	-	-
N5PMPB180	FALSE	-	-	-	-	-	-	-	-
Mab504 (+ve Control)	TRUE	14.24 ± 0.02	2.16 ± 0.44	1.08 ± 0.06	0.82 ± 0.01	FALSE	FALSE	FALSE	FALSE
MGO53 (-ve Control)	TRUE	0.86 ± 0.15	1.47 ± 0.43	0.89 ± 0.11	0.78 ± 0.04	FALSE	FALSE	FALSE	FALSE
ED38	TRUE	5.98 ± 0.15	13.85 ± 0.05	3.37 ± 0.07	4.22 ± 0.37	FALSE	FALSE	FALSE	FALSE
JB40	TRUE	3.39 ± 0.04	8.97 ± 0.40	1.33 ± 0.06	3.38 ± 0.09	FALSE	FALSE	FALSE	FALSE
PBS	-----	0.51 ± 0.03	0.40 ± 0.10	0.51 ± 0.09	0.59 ± 0.09	FALSE	FALSE	FALSE	FALSE

Table 25: Vax3 monoclonal antibody reactivity data

ANTIBODY	EXPRESSED	Bexsero (AUC±SEM)	Insulin (AUC±SEM)	LPS (AUC±SEM)	dsDNA (AUC±SEM)	fHbp	NadA	NHBA	PorA P1.4
N6PMPB091K	TRUE	12.08 ± 1.27	0.76 ± 0.15	0.53 ± 0.12	0.59 ± 0.11	FALSE	FALSE	TRUE	FALSE
N6PMPB091L	TRUE	2.60 ±0.59	1.11 ± 0.14	0.79 ± 0.24	0.68 ± 0.07	FALSE	FALSE	TRUE	FALSE
N6PMPB114	TRUE	3.57 ± 0.37	1.61 ± 0.16	0.68 ± 0.238	0.60 ± 0.12	FALSE	FALSE	TRUE	FALSE
N6PMPB123	TRUE	0.66 ± 0.09	0.85 ± 0.06	0.89 ± 0.19	0.69 ± 0.03	FALSE	FALSE	FALSE	FALSE
N6PMPB126	FALSE	-	-	-	-	-	-	-	-
N6PMPB126	TRUE	0.78 ± 0.2	1.21 ± 0.20	0.61 ± 0.07	0.59 ± 0.11	FALSE	FALSE	FALSE	FALSE
N6PMPB127	TRUE	0.79 ± 0.20	1.17 ± 0.03	0.48 ± 0.07	0.53 ± 0.10	FALSE	FALSE	FALSE	FALSE
N6PMPB134	TRUE	3.31 ± 0.57	3.46 ± 0.20	1.22 ± 0.30	0.92 ± 0.06	FALSE	FALSE	FALSE	FALSE
N6PMPB135	TRUE	0.84 ± 0.22	1.18 ± 0.08	0.53 ± 0.10	0.73 ± 0.16	FALSE	FALSE	TRUE	FALSE
N6PMPB135	TRUE	0.84 ± 0.22	1.18 ± 0.08	0.53 ± 0.10	0.73 ± 0.16	FALSE	FALSE	TRUE	FALSE
N6PMPB138	TRUE	1.75 ± 0.64	0.63 ± 0.05	0.43 ± 0.04	0.50 ± 0.07	FALSE	FALSE	TRUE	FALSE
N6PMPB147K	TRUE	1.90 ± 0.37	1.43 ± 0.35	1.13 ± 0.37	1.47 ± 0.43	FALSE	FALSE	FALSE	FALSE
N6PMPB147L	TRUE	1.74 ± 0.12	1.62 ± 0.09	0.55 ± 0.03	1.21 ± 0.08	FALSE	FALSE	FALSE	FALSE
N6PMPB150K	FALSE	-	-	-	-	-	-	-	-
N6PMPB150L	FALSE	-	-	-	-	-	-	-	-
N6PMPB153	TRUE	1.21 ± 0.43	1.74 ± 0.31	0.90 ± 0.15	0.81 ± 0.17	FALSE	FALSE	FALSE	FALSE
N6PMPB158K	FALSE	-	-	-	-	-	-	-	-
N6PMPB158L	FALSE	-	-	-	-	-	-	-	-
N6PMPB196	FALSE	-	-	-	-	-	-	-	-
N6PMPB224	TRUE	7.93 ± 1.29	1.29 ± 0.42	1.39 ± 0.58	0.91 ± 0.18	FALSE	FALSE	TRUE	FALSE
N6PMPB267	TRUE	2.50 ± 0.24	0.95 ± 0.08	0.36 ± 0.01	0.79 ± 0.03	FALSE	FALSE	TRUE	FALSE
N6PMPB298	TRUE	1.64 ± 0.10	0.88 ± 0.06	0.41 ± 0.04	0.79 ± 0.09	FALSE	FALSE	FALSE	FALSE
N6PMPB299	TRUE	0.70 ± 0.20	0.81 ± 0.09	0.48 ± 0.08	0.58 ± 0.08	FALSE	FALSE	FALSE	FALSE
N6PMPB303	TRUE	1.31 ± 0.21	3.16 ± 0.25	1.04 ± 0.23	0.78 ± 0.08	FALSE	FALSE	FALSE	FALSE
N6PMPB304	FALSE	-	-	-	-	-	-	-	-
N6PMPB328K	TRUE	0.68 ± 0.06	0.77 ± 0.05	0.76 ± 0.16	0.73 ± 0.08	FALSE	FALSE	FALSE	FALSE
N6PMPB328L	FALSE	-	-	-	-	-	-	-	-
N6PMPB346K	TRUE	0.97 ± 0.12	2.45 ± 0.29	0.82 ± 0.17	0.88 ± 0.11	FALSE	FALSE	FALSE	FALSE
N6PMPB346L	TRUE	1.47 ± 0.17	0.83 ± 0.06	0.80 ± 0.29	0.73 ± 0.07	FALSE	FALSE	TRUE	FALSE
N6PMPB371	TRUE	3.40 ± 0.70	1.41 ± 0.41	0.76 ± 0.23	0.79 ± 0.12	FALSE	FALSE	TRUE	FALSE
N6PMPB372	TRUE	0.66 ± 0.20	0.64 ± 0.03	0.44 ± 0.03	0.5 ± 0.04	FALSE	FALSE	FALSE	FALSE
N6PMPB069	TRUE	0.70 ± 0.18	0.88 ± 0.06	0.81 ± 0.23	0.72 ± 0.11	FALSE	FALSE	FALSE	FALSE
N6PMPB101	TRUE	0.34 ± 0.03	0.57 ± 0.03	0.37 ± 0.02	0.45 ± 0.09	FALSE	FALSE	FALSE	FALSE
N6PMPB104	TRUE	0.67 ± 0.17	0.83 ± 0.06	0.46 ± 0.05	0.57 ± 0.07	FALSE	FALSE	FALSE	FALSE
N6PMPB117	TRUE	0.52 ± 0.10	0.89 ± 0.16	0.75 ± 0.25	0.54 ± 0.03	FALSE	FALSE	FALSE	FALSE
N6PMPB118	TRUE	-	-	-	-	-	-	-	-
N6PMPB128	TRUE	-	-	-	-	-	-	-	-
N6PMPB142	TRUE	0.92 ± 0.17	1.80 ± 0.24	0.82 ± 0.09	0.63 ± 0.07	FALSE	FALSE	FALSE	FALSE
N6PMPB148	TRUE	13.64 ± 0.31	1.85 ± 0.34	0.76 ± 0.11	0.76 ± 0.15	FALSE	FALSE	TRUE	FALSE
N6PMPB161	TRUE	9.27 ± 1.39	1.26 ± 0.56	0.48 ± 0.06	0.60 ± 0.05	FALSE	FALSE	TRUE	FALSE

Table 25 (Continued)..

ANTIBODY	EXPRESSED	Bexsero (AUC±SEM)	Insulin (AUC±SEM)	LPS (AUC±SEM)	dsDNA (AUC±SEM)	fHbp	NadA	NHBA	PorA P1.4
N6PMPB174	TRUE	4.80 ± 0.63	8.25 ± 1.24	1.85 ± 0.26	3.03 ± 0.18	FALSE	FALSE	FALSE	FALSE
N6PMPB177	TRUE	0.83 ± 0.19	3.13 ± 0.21	0.70 ± 0.08	0.73 ± 0.17	FALSE	FALSE	FALSE	FALSE
N6PMPB181	TRUE	3.67 ± 0.55	2.32 ± 0.38	1.08 ± 0.37	1.16 ± 0.20	FALSE	FALSE	FALSE	FALSE
N6PMPB198	TRUE	1.56 ± 0.26	3.82 ± 0.84	2.25 ± 0.71	1.03 ± 0.13	FALSE	FALSE	FALSE	FALSE
N6PMPB201	FALSE	-	-	-	-	-	-	-	-
N6PMPB211	TRUE	-	-	-	-	-	-	-	-
N6PMPB214	TRUE	1.89 ± 0.21	3.59 ± 1.80	1.54 ± 0.20	2.16 ± 0.55	FALSE	FALSE	TRUE	FALSE
N6PMPB218	FALSE	-	-	-	-	-	-	-	-
N6PMPB223	FALSE	-	-	-	-	-	-	-	-
N6PMPB226	TRUE	0.56 ± 0.06	0.64 ± 0.06	0.64 ± 0.17	0.62 ± 0.03	FALSE	FALSE	FALSE	FALSE
N6PMPB247	TRUE	1.88 ± 0.28	3.50 ± 0.21	2.08 ± 0.52	1.15 ± 0.25	FALSE	FALSE	FALSE	FALSE
N6PMPB253	TRUE	0.43 ± 0.02	0.73 ± 0.12	0.49 ± 0.04	0.60 ± 0.03	FALSE	FALSE	FALSE	FALSE
N6PMPB255	FALSE	-	-	-	-	-	-	-	-
N6PMPB258	TRUE	2.26 ± 0.46	1.77 ± 0.38	1.31 ± 0.16	0.80 ± 0.04	TRUE	FALSE	FALSE	FALSE
N6PMPB260	TRUE	0.55 ± 0.08	0.90 ± 0.10	0.91 ± 0.24	0.59 ± 0.01	FALSE	FALSE	FALSE	FALSE
N6PMPB262	TRUE	0.36 ± 0.04	0.53 ± 0.11	0.38 ± 0.03	0.39 ± 0.06	FALSE	FALSE	FALSE	FALSE
N6PMPB268	TRUE	0.66 ± 0.18	0.75 ± 0.18	0.67 ± 0.06	0.57 ± 0.14	FALSE	FALSE	FALSE	FALSE
N6PMPB272	FALSE	-	-	-	-	-	-	-	-
N6PMPB273	TRUE	0.77 ± 0.13	0.93 ± 0.02	0.35 ± 0.03	0.46 ± 0.025	FALSE	FALSE	FALSE	FALSE
N6PMPB277	TRUE	2.55 ± 1.07	6.50 ± 3.29	2.07 ± 0.91	2.05 ± 0.86	FALSE	FALSE	FALSE	FALSE
N6PMPB283	FALSE	-	-	-	-	-	-	-	-
N6PMPB294	TRUE	1.08 ± 0.52	1.38 ± 0.34	0.80 ± 0.23	0.63 ± 0.02	FALSE	FALSE	FALSE	FALSE
N6PMPB297	TRUE	0.82 ± 0.16	2.05 ± 0.19	0.63 ± 0.16	0.53 ± 0.04	FALSE	FALSE	FALSE	FALSE
N6PMPB302	TRUE	1.65 ± 0.19	3.80 ± 0.16	2.05 ± 0.55	1.06 ± 0.31	FALSE	FALSE	FALSE	FALSE
N6PMPB318	TRUE	1.29 ± 0.17	2.83 ± 1.39	1.63 ± 0.43	1.42 ± 0.44	FALSE	FALSE	TRUE	FALSE
N6PMPB321	TRUE	1.81 ± 0.23	0.89 ± 0.05	0.42 ± 0.02	1.10 ± 0.16	FALSE	FALSE	FALSE	FALSE
N6PMPB339	TRUE	1.34 ± 0.24	5.30 ± 0.17	1.52 ± 0.48	1.01 ± 0.07	FALSE	FALSE	FALSE	FALSE
N6PMPB340	TRUE	-	-	-	-	-	-	-	-
N6PMPB343	TRUE	-	-	-	-	-	-	-	-
N6PMPB344	TRUE	1.16 ± 0.23	0.81 ± 0.13	0.37 ± 0.01	0.64 ± 0.01	FALSE	FALSE	-	FALSE
N6PMPB345	TRUE	4.77 ± 0.35	5.62 ± 1.49	2.61 ± 0.62	2.61 ± 0.73	FALSE	FALSE	TRUE	FALSE
N6PMPB349	TRUE	1.27 ± 0.38	3.19 ± 0.34	1.32 ± 0.59	0.88 ± 0.11	FALSE	FALSE	-	FALSE
N6PMPB350	TRUE	1.25 ± 0.15	2.49 ± 1.05	1.07 ± 0.26	1.36 ± 0.36	FALSE	FALSE	TRUE	FALSE
N6PMPB355	TRUE	13.69 ± 0.15	4.03 ± 1.13	1.75 ± 0.47	1.31 ± 0.31	FALSE	FALSE	TRUE	FALSE
N6PMPB361	TRUE	12.47 ± 0.76	3.53 ± 1.03	1.80 ± 0.39	1.98 ± 0.54	FALSE	FALSE	TRUE	FALSE
N6PMPB365	TRUE	0.94 ± 0.14	1.13 ± 0.06	0.51 ± 0.10	0.67 ± 0.24	FALSE	FALSE	-	FALSE
N6PMPB369	TRUE	14.62 ± 0.15	7.03 ± 0.29	3.92 ± 0.85	2.46 ± 0.58	FALSE	FALSE	TRUE	FALSE
N6PMPB370	FALSE	-	-	-	-	-	-	-	-
N6PMPB373	TRUE	7.45 ± 1.47	0.91 ± 0.11	0.92 ± 0.50	0.78 ± 0.19	FALSE	FALSE	TRUE	FALSE
N6PMPB374	FALSE	-	-	-	-	-	-	-	-
N6PMPB377	TRUE	12.23 ± 0.82	2.83 ± 0.92	1.74 ± 0.58	1.66 ± 0.52	FALSE	FALSE	TRUE	FALSE

Table 25 (Continued).....

ANTIBODY	EXPRESSED	Bexsero (AUC±SEM)	Insulin (AUC±SEM)	LPS (AUC±SEM)	dsDNA (AUC±SEM)	fHbp	NadA	NHBA	PorA P1.4
N6PMPB384	TRUE	14.01 ± 0.15	2.05 ± 0.40	1.07 ± 0.41	0.71 ± 0.03	FALSE	FALSE	TRUE	FALSE
N6PMPB008	TRUE	1.28 ± 0.17	0.66 ± 0.11	0.43 ± 0.04	0.39 ± 0.01	FALSE	FALSE	TRUE	FALSE
N6PMPB013	TRUE	5.81 ± 1.14	0.83 ± 0.17	0.90 ± 0.27	0.60 ± 0.02	FALSE	TRUE	FALSE	FALSE
N6PMPB015	TRUE	0.54 ± 0.05	1.07 ± 0.23	0.60 ± 0.08	0.72 ± 0.11	FALSE	FALSE	FALSE	FALSE
N6PMPB033	TRUE	5.65 ± 1.04	0.82 ± 0.09	0.55 ± 0.03	0.50 ± 0.08	TRUE	FALSE	FALSE	FALSE
N6PMPB100	TRUE	0.80 ± 0.15	1.21 ± 0.33	0.66 ± 0.19	0.68 ± 0.11	FALSE	FALSE	FALSE	FALSE
N6PMPB102	FALSE	-	-	-	-	-	-	-	-
N6PMPB105	TRUE	0.50 ± 0.06	0.68 ± 0.11	1.11 ± 0.25	0.45 ± 0.05	FALSE	FALSE	FALSE	FALSE
N6PMPB112	TRUE	0.40 ± 0.05	0.68 ± 0.12	0.42 ± 0.04	0.41 ± 0.06	FALSE	FALSE	FALSE	FALSE
N6PMPB113	TRUE	0.82 ± 0.10	2.07 ± 0.92	0.85 ± 0.19	0.79 ± 0.13	FALSE	FALSE	FALSE	FALSE
N6PMPB115	FALSE	-	-	-	-	-	-	-	-
N6PMPB124	TRUE	1.33 ± 0.20	2.17 ± 0.45	1.63 ± 0.41	0.90 ± 0.12	FALSE	FALSE	FALSE	FALSE
N6PMPB125	FALSE	-	-	-	-	-	-	-	-
N6PMPB129	FALSE	-	-	-	-	-	-	-	-
N6PMPB137	FALSE	-	-	-	-	-	-	-	-
N6PMPB159	TRUE	0.59 ± 0.05	0.81 ± 0.09	0.70 ± 0.15	0.63 ± 0.03	FALSE	FALSE	FALSE	FALSE
N6PMPB162	TRUE	0.48 ± 0.12	0.44 ± 0.06	0.40 ± 0.04	0.42 ± 0.04	FALSE	FALSE	FALSE	FALSE
N6PMPB166	TRUE	0.61 ± 0.07	0.72 ± 0.06	0.76 ± 0.21	0.68 ± 0.06	FALSE	FALSE	FALSE	FALSE
N6PMPB171	TRUE	6.27 ± 0.61	0.96 ± 0.27	1.17 ± 0.53	0.71 ± 0.07	FALSE	TRUE	FALSE	FALSE
N6PMPB175	TRUE	0.56 ± 0.07	1.37 ± 0.54	0.55 ± 0.08	0.65 ± 0.09	FALSE	FALSE	FALSE	FALSE
N6PMPB179	TRUE	1.31 ± 0.19	3.35 ± 0.56	1.69 ± 0.76	1.44 ± 0.23	FALSE	FALSE	FALSE	FALSE
N6PMPB190	TRUE	1.28 ± 0.24	0.67 ± 0.17	0.46 ± 0.01	0.41 ± 0.04	FALSE	FALSE	TRUE	FALSE
N6PMPB194	TRUE	0.35 ± 0.02	0.37 ± 0.03	0.32 ± 0.02	0.33 ± 0.01	FALSE	FALSE	FALSE	FALSE
N6PMPB250	TRUE	0.79 ± 0.11	1.36 ± 0.52	0.68 ± 0.21	0.66 ± 0.15	FALSE	FALSE	FALSE	FALSE
N6PMPB274	FALSE	-	-	-	-	-	-	-	-
N6PMPB275	FALSE	-	-	-	-	-	-	-	-
N6PMPB276	FALSE	-	-	-	-	-	-	-	-
N6PMPB279	TRUE	4.85 ± 1.20	2.43 ± 0.71	2.19 ± 0.53	1.12 ± 0.20	FALSE	FALSE	FALSE	FALSE
N6PMPB314	TRUE	0.51 ± 0.02	0.61 ± 0.14	0.52 ± 0.09	0.50 ± 0.07	FALSE	FALSE	FALSE	FALSE
N6PMPB325	TRUE	3.01 ± 1.07	7.76 ± 0.89	3.45 ± 0.95	4.94 ± 0.50	FALSE	FALSE	FALSE	FALSE

Table 25 (Continued).....

N6PMPB331	TRUE	0.67 ± 0.22	0.68 ± 0.06	0.45 ± 0.05	0.53 ± 0.04	FALSE	FALSE	FALSE	FALSE
N6PMPB334	TRUE	0.42 ± 0.08	0.79 ± 0.14	0.43 ± 0.02	0.51 ± 0.06	FALSE	FALSE	FALSE	FALSE
N6PMPB342	FALSE	-	-	-	-	-	-	-	-
N6PMPB354	TRUE	0.40 ± 0.03	0.69 ± 0.12	0.53 ± 0.05	0.56 ± 0.01	FALSE	FALSE	FALSE	FALSE
N6PMPB356	TRUE	0.73 ± 0.21	0.69 ± 0.05	0.46 ± 0.06	0.56 ± 0.06	FALSE	FALSE	FALSE	FALSE
N6PMPB367	TRUE	0.70 ± 0.18	0.76 ± 0.04	0.51 ± 0.05	0.57 ± 0.11	FALSE	FALSE	FALSE	FALSE
N6PMPB368	TRUE	0.53 ± 0.08	0.68 ± 0.09	0.65 ± 0.17	0.59 ± 0.05	FALSE	FALSE	FALSE	FALSE
Mab504 (+ve Control)	TRUE	13.25 ± 0.39	2.02 ± 0.10	1.01 ± 0.07	0.78 ± 0.07	FALSE	FALSE	FALSE	FALSE
MGO53 (-ve Control)	TRUE	0.76 ± 0.14	1.41 ± 0.16	0.81 ± 0.09	0.73 ± 0.04	FALSE	FALSE	FALSE	FALSE
ED38	TRUE	3.56 ± 0.48	11.67 ± 0.25	3.11 ± 0.38	5.05 ± 0.16	FALSE	FALSE	FALSE	FALSE
JB40	TRUE	1.89 ± 0.30	6.28 ± 0.41	1.59 ± 0.15	4.88 ± 0.23	FALSE	FALSE	FALSE	FALSE
PBS	-----	0.46 ± 0.04	0.52 ± 0.03	0.50 ± 0.04	0.57 ± 0.05	FALSE	FALSE	FALSE	FALSE

Table 26: Vax4 monoclonal antibody reactivity data

ANTIBODY	EXPRESSED	Bexsero (AUC+-SEM)	Insulin (AUC+-SEM)	LPS (AUC+-SEM)	dsDNA (AUC+-SEM)	fHbp	NadA	NHBA	PorA P1.4
N7PMPB206K	TRUE	0.59 ± 0.06	0.81 ± 0.09	0.65 ± 0.04	0.55 ± 0.05	FALSE	FALSE	FALSE	FALSE
N7PMPB206L	FALSE	-	-	-	-	-	-	-	-
N7PMPB231K	FALSE	-	-	-	-	-	-	-	-
N7PMPB235K	TRUE	1.09 ± 0.08	2.79 ± 0.53	1.22 ± 0.29	1.21 ± 0.34	FALSE	FALSE	FALSE	FALSE
N7PMPB235L	TRUE	1.12 ± 0.04	1.90 ± 0.42	1.22 ± 0.35	0.85 ± 0.09	FALSE	FALSE	FALSE	FALSE
N7PMPB241K	FALSE	-	-	-	-	-	-	-	-
N7PMPB241L	TRUE	1.10 ± 0.03	1.05 ± 0.11	1.36 ± 0.27	0.67 ± 0.10	FALSE	FALSE	FALSE	FALSE
N7PMPB255	TRUE	0.96 ± 0.28	0.55 ± 0.05	1.23 ± 0.41	1.07 ± 0.29	FALSE	FALSE	FALSE	FALSE
N7PMPB258	TRUE	0.94 ± 0.32	1.11 ± 0.12	0.95 ± 0.41	0.70 ± 0.10	FALSE	FALSE	FALSE	FALSE
N7PMPB230	TRUE	0.59 ± 0.05	0.88 ± 0.13	0.70 ± 0.20	0.60 ± 0.07	FALSE	FALSE	FALSE	FALSE
N7PMPB236	TRUE	1.28 ± 0.27	1.24 ± 0.09	1.62 ± 0.30	0.77 ± 0.11	FALSE	FALSE	FALSE	FALSE
N7PMPB242	TRUE	0.85 ± 0.05	1.41 ± 0.32	0.95 ± 0.39	0.75 ± 0.17	FALSE	FALSE	FALSE	FALSE
N7PMPB245	TRUE	1.06 ± 0.10	1.63 ± 0.34	0.97 ± 0.20	0.72 ± 0.03	FALSE	FALSE	FALSE	FALSE
N7PMPB252	FALSE	-	-	-	-	-	-	-	-
N7PMPB265	FALSE	-	-	-	-	-	-	-	-
N7PMPB275	FALSE	-	-	-	-	-	-	-	-
N7PMPB276	TRUE	0.90 ± 0.32	0.78 ± 0.05	0.99 ± 0.30	0.59 ± 0.03	FALSE	FALSE	FALSE	FALSE
N7PMPB281	TRUE	1.69 ± 0.25	3.27 ± 0.81	2.08 ± 0.79	1.20 ± 0.43	FALSE	FALSE	FALSE	FALSE
N7PMPB283	FALSE	-	-	-	-	-	-	-	-

Table 26 (Continued).....

N7PMPB207	TRUE	0.85 ± 0.05	1.54 ± 0.38	1.48 ± 0.78	0.80 ± 0.14	FALSE	FALSE	FALSE	FALSE
N7PMPB216	FALSE	-	-	-	-	-	-	-	-
N7PMPB248	FALSE	-	-	-	-	-	-	-	-
N7PMPB262	TRUE	1.15 ± 0.21	1.19 ± 0.16	1.37 ± 0.24	0.87 ± 0.24	FALSE	FALSE	FALSE	FALSE
N7PMPB196	TRUE	1.44 ± 0.67	0.91 ± 0.06	0.91 ± 0.06	0.62 ± 0.10	FALSE	FALSE	FALSE	FALSE
N7PMPB237	FALSE	-	-	-	-	-	-	-	-
N7PMPB261	TRUE	0.72 ± 0.14	0.68 ± 0.05	0.68 ± 0.05	0.55 ± 0.03	FALSE	FALSE	FALSE	FALSE
N7PMPB270	FALSE	-	-	-	-	-	-	-	-
N7PMPB287	FALSE	-	-	-	-	-	-	-	-
N7PMPB205	FALSE	-	-	-	-	-	-	-	-
N7PMPB218	FALSE	-	-	-	-	-	-	-	-
N7PMPB226	FALSE	-	-	-	-	-	-	-	-
N7PMPB228	TRUE	1.16 ± 0.16	1.83 ± 0.47	1.74 ± 0.57	0.77 ± 0.11	FALSE	FALSE	FALSE	FALSE
N7PMPB259	TRUE	0.51 ± 0.03	0.72 ± 0.12	0.58 ± 0.11	0.51 ± 0.06	FALSE	FALSE	FALSE	FALSE
N7PMPB267	FALSE	-	-	-	-	-	-	-	-
N7PMPB269	FALSE	-	-	-	-	-	-	-	-
Mab504 (+ve Control)	TRUE	14.10 ± 0.26	1.59 ± 0.33	1.41 ± 0.18	0.72 ± 0.16	FALSE	FALSE	FALSE	FALSE
MGO53 (-ve Control)	TRUE	0.76 ± 0.08	1.21 ± 0.30	0.98 ± 0.31	0.81 ± 0.26	FALSE	FALSE	FALSE	FALSE
ED38	TRUE	3.89 ± 0.59	11.26 ± 0.09	2.90 ± 0.61	3.12 ± 0.48	FALSE	FALSE	FALSE	FALSE
JB40	TRUE	1.98 ± 0.34	6.78 ± 0.67	1.84 ± 0.39	2.17 ± 0.21	FALSE	FALSE	FALSE	FALSE
PBS	-----	0.44 ± 0.06	0.49 ± 0.05	0.45 ± 0.05	0.53 ± 0.09	FALSE	FALSE	FALSE	FALSE

Table 27: Vax2 Polyreactive antibodies

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N5PMPB045	IGHV3-7*01	IGHD6-25*01	IGHJ4*02	TYRSSGTDY	9	14	IGHG2	IGKV2-30*01	IGKJ3*01	VQLTQWPFT	7	IGKC					
N5PMPB112	IGHV3-23*04	IGHD4-17*01	IGHJ3*02	KLHDYVQGGAFDI	13	25	IGHG1	IGKV1-5*03	IGKJ2*01	QQYYSYLYT	14	IGKC					
N5PMPB029	IGHV1-69*01	IGHD4-17*01	IGHJ5*02	GGWTISTESTRGHLEQ	17	31	IGHA1						IGLV8-61*01	IGLJ3*02	VLYLGSYNWV	13	
N5PMPB085	IGHV3-30*14	IGHD3-10*02	IGHJ5*02	DKGNYYTSGSHFRVLDS	17	33	IGHG1	IGKV3-20*01	IGKJ5*01	QQYYDSPPIT	30	IGKC					
N5PMPB023	IGHV3-53*01	IGHD2-15*01	IGHJ4*02	FCSAGTCDSEEAFDY	16	22	IGHA1						IGLV2-23*02	IGLJ1*01	FSYARGDSYV	21	
N5PMPB134	IGHV4-34*01	IGHD3-10*01	IGHJ4*02	GRGKLQWFAELYH	13	4	IGHG1	IGKV3-20*01	IGKJ2*01	QQYGSSPRT	5	IGKC					
N5PMPB164	IGHV4-39*01	IGHD4-17*01	IGHJ2*01	HATVTAGGDFDL	13	10	IGHA1						IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB111	IGHV3-7*01	IGHD6-19*01	IGHJ5*02	RMVAVIGTGTFA	12	17	IGHG1	IGKV4-1*01	IGKJ1*01	LQNYNGRWT	23	IGKC					
N5PMPB062	IGHV1-46*01	IGHD5-24*01	IGHJ5*02	WLKGDETDH	9	13	IGHA1	IGKV1-17*01	IGKJ1*01	LQHDTYPWT	13	IGKC					
N5PMPB074	IGHV1-69*06	IGHD3-16*02	IGHJ5*02	DQVPWLGAEVPVALES	16	45	IGHG3	IGKV3-20*01	IGKJ4*01	HQYGDSPLT	25	IGKC					
N5PMPB045	IGHV3-7*01	IGHD6-25*01	IGHJ4*02	TYRSSGTDY	9	14	IGHG2	IGKV2-30*01	IGKJ3*01	VQLTQWPFT	7	IGKC					
N5PMPB131	IGHV3-30*18	IGHD6-19*01	IGHJ4*02	GNAMGPHGCFDY	12	11	IGHG1	IGKV1-39*01	IGKJ4*01	QQSFRTPLT	14	IGKC					
N5PMPB013	IGHV4-34*01	IGHD3-9*01	IGHJ6*03	GRRYFDWPTFVMDV	14	1	IGHM	IGKV1-17*01	IGKJ4*01	LQHNSYPLT	6	IGKC					
N5PMPB118	IGHV3-9*01	IGHD3-16*02	IGHJ2*01	NGGGSTWHWYFDL	13	16	IGHG1	IGKV3D-15*01	IGKJ3*01	QQYNNWPLS	5	IGKC					
N5PMPB156	IGHV1-46*02	IGHD1-26*01	IGHJ5*02	DYSHTNWLGP	10	30	IGHA1	IGKV3-20*01	IGKJ2*02	QHYDHSPRVYT	23	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB136	IGHV3-23*04	IGHD6-19*01	IGHJ5*01	LQSSGWYSDL	10	19	IGHG1	IGKV1-39*01	IGKJ2*01	QQSYNTYT	10	IGKC					
N5PMPB127	IGHV1-46*02	IGHD3-10*01	IGHJ6*02	DLTMVRGLVGYYGMDV	16	24	IGHG1	IGKV2-28*01	IGKJ2*01	MQALQTPPYT	6	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1
N5PMPB037	IGHV3-7*01	IGHD5-24*01	IGHJ4*02	AKHGY	5	0	IGHG1	IGKV3-11*01	IGKJ4*02	QQRSNWPWT	3	IGKC					
N5PMPB153	IGHV3-43*01	IGHD3-3*01	IGHJ6*02	GGYYDFWSGYDKRPYHYYGMDV	23	5	IGHA1						IGLV2-14*01	IGLJ1*01	SSYTGSSTDV	2	IGLC1
N5PMPB014	IGHV1-2*02	IGHD2-2*02	IGHJ4*01	GYSDIIGRCLDY	12	15	IGHA2	IGKV1-9*01	IGKJ1*01	QQLNSYPRT	4	IGKC					
N5PMPB012	IGHV3-9*01	IGHD5-5*01	IGHJ4*02	DIHPLKIQLWLGGFDY	16	7	IGHG1						IGLV2-23*03	IGLJ1*01	CSYAGSSTYV	3	
N5PMPB025	IGHV3-21*01	IGHD1-14*01	IGHJ6*03	VANLRANRYYYYYMDV	16	0	IGHM	IGKV3-11*01	IGKJ2*01	QQRSNWLYT	3	IGKC					
N5PMPB056	IGHV4-4*07	IGHD2-2*02	IGHJ5*02	GGYFSTSGSNWFDP	15	7	IGHM						IGLV2-8*02	IGLJ1*01	SSYAGSNIYV	1	
N5PMPB081	IGHV3-48*02	IGHD6-19*01	IGHJ4*02	SGWSRYHFDH	10	10	IGHG1	IGKV3-20*01	IGKJ1*01	QQYGSSLWT	6	IGKC					
N5PMPB003	IGHV3-23*04	IGHD3-22*01	IGHJ4*02	DRYSTASYYFDY	12	31	IGHA1	IGKV1-12*01	IGKJ2*02	QLPNSLPPIYA	24	IGKC					
N5PMPB088	IGHV3-9*01	IGHD2-2*02	IGHJ1*01	ESPPCTNTWSSFHR	14	22	IGHG2	IGKV3-20*01	IGKJ4*02	HQYGSSPLT	13	IGKC					

Table 28: Vax4 Polyreactive antibodies

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N7PMPB261	IGHV3-23*01	IGHD1-1*01	IGHJ6*02	EYRLLTTATHSMDV	14	12	IGHA2						IGLV2-14*01	IGLJ1*01	FSYTITSTPYV	8	IGLC1
N7PMPB228	IGHV3-23*01	IGHD2-21*02	IGHJ4*02	DRFRNKYEDYQYCGGDCRSPPPPDY	26	5	IGHG2						IGLV1-44*01	IGLJ3*02	AWDDSLNGWV	4	IGLC1
N7PMPB259	IGHV4-31*03	IGHD3-10*02	IGHJ2*01	APYNDGLPGFGYFDL	16	25	IGHG4						IGLV3-21*02	IGLJ2*01	QVWDSSSDRVV	0	IGLC7
N7PMPB258	IGHV4-59*01	IGHD3-9*01	IGHJ4*02	GTPSYDILTGYETAYFDY	18	0	IGHM	IGKV3-20*01	IGKJ5*01	QQYGSSPIT	4	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N7PMPB241	IGHV3-30*18	IGHD2-2*02	IGHJ5*02	DRYPGEILIWFDP	13	6	IGHA1	GKV2D-26*0	IGKJ3*01	MQDSQDPLFT	8	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	7	IGLC1
N7PMPB276	IGHV4-39*01	IGHD6-19*01	IGHJ4*02	RLAVGWPRDYFDY	13	24	IGHG1	IGKV1-12*01	IGKJ1*01	QQAHSFPWT	12	IGKC					
N7PMPB206	IGHV3-30*18	IGHD3-10*02	IGHJ5*02	DRYPGEILIWFDP	13	7	IGHA1	GKV2D-26*0	IGKJ3*01	MQDSQDPLFT	10	IGKC	IGLV2-23*02	IGLJ1*01	CSYAGSYTFV	8	IGLC1
N7PMPB236	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	18	IGKC					
N7PMPB262	IGHV4-59*01	IGHD3-3*01	IGHJ4*02	APNDLWSGYSDY	12	22	IGHG1	IGKV1-27*01	IGKJ1*01	QKYNFAPWT	13	IGKC					
N7PMPB245	IGHV3-74*01	IGHD6-19*01	IGHJ4*02	DTRSGWYGGFDY	12	0	IGHM	IGKV4-1*01	IGKJ2*01	QQYYSTPYT	1	IGKC					
N5PMPB182	IGHV1-8*01	IGHD3-16*02	IGHJ5*02	GAGEGGRDWFDP	12	20	IGHA2	IGKV4-1*01	IGKJ4*01	QQYHSKPPT	11	IGKC					
N7PMPB196	IGHV1-18*01	IGHD6-13*01	IGHJ6*02	EGGVPPEGQQLHILRGYYYYGMDV	25	1	IGHG1	IGLV1-36*01	IGLJ3*02	AAWDDSLNGP	2	IGLC7					
N7PMPB255	IGHV3-74*03	IGHD3-10*02	IGHJ3*02	DRGWNALDL	9	14	IGHA2	IGKV2-29*02	IGKJ3*01	MQGLHLPLT	11	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	15	IGLC1
N7PMPB281	IGHV5-51*01	IGHD6-13*01	IGHJ3*02	VRVAAAGRGMYVGNAFDI	18	0	IGHM	IGKV3-20*01	IGKJ3*01	QQYGSSPLFT	4	IGKC					
N7PMPB242	IGHV5-10-1*01	IGHD1-7*01	IGHJ4*03	VPWYNSSTWFAPNW	14	22	IGHA2	IGKV1-16*02	IGKJ4*01	QQYKSYPET	11	IGKC					
N7PMPB235	IGHV3-64*01	IGHD2-15*01	IGHJ4*02	GGYCSGGSCYLPY	13	0	IGHG1	IGKV4-1*01	IGKJ1*01	QQYYSTPQT	1	IGKC	IGLV1-44*01	IGLJ1*01	AAWDDSLNGRV	14	IGLC1

Table 29: Vax3 Polyreactive antibodies

HEAVY CHAIN								KAPPA CHAIN					LAMBDA CHAIN				
ANTIBODY NAME	V	D	J	CDR3	CDR3 Length	SHM	Constant	V	D	CDR3	SHM	Constant	V	J	CDR3	SHM	Constant
N6PMPB349	IGHV5-51*01	IGHD3-3*02	IGHJ5*02	RAYGGSELATNNWFDP	16	36	IGHG2	IGKV2-30*01	IGKJ1*01	MQRSRRPWA	32	IGKC					
N6PMPB015	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DQNPLLWFGGSLDY	14	10	IGHG4						IGLV1-44*01	IGLJ3*01	AAWDDSLNAHR	1	IGLC7
N6PMPB177	IGHV3-9*01	IGHD3-10*01	IGHJ6*02	DRVTMVRGYGMDV	13	2	IGHG2	IGKV4-1*01	IGKJ2*01	QQHHSTPYT	12	IGKC					
N6PMPB134	IGHV4-59*01	IGHD3-22*01	IGHJ5*02	GPTMIQE	7	1	IGHG3	IGKV1-5*01	IGKJ2*01	QHYKTYSPT	12	IGKC					
N6PMPB198	IGHV4-39*01	IGHD3-3*02	IGHJ4*02	RPFSALLEWLFTPFDN	16	17	IGHG4	IGKV1-5*03	IGKJ3*01	QQYNSYLFT	14	IGKC					
N6PMPB147L	IGHV4-4*07	IGHD3-10*01	IGHJ6*02	AGAVLTPLWFGETHYYYYPMDV	22	13	IGHG1						IGLV3-21*01	IGLJ3*02	QVWHTSSDHRGV	26	IGLC3
N6PMPB105	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	SGAWWAFDY	9	22							IGLV2-8*01	IGLJ3*02	SSYGGSANVL	10	IGLC7
N6PMPB279	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DSYGSGIYRRMDN	13	35	IGHA1						IGLV2-11*01	IGLJ1*01	CSFAGKFTYV	24	IGLC1
N6PMPB303	IGHV1-8*01	IGHD3-10*01	IGHJ6*02	AWDVEDREDFGMDV	14	23	IGHA1	IGKV1D-39*01	IGKJ1*01	QQSSTTPWT	18	IGKC					
N6PMPB113	IGHV3-11*01	IGHD3-22*01	IGHJ6*02	NRGGFYIYGMDV	12	17	IGHG1						IGLV6-57*01	IGLJ3*01	QSYDSSDVV	9	
N6PMPB181	IGHV4-39*07	IGHD3-10*02	IGHJ4*02	GTSFHYGSGSFYKQPPFDS	19	33	IGHG1	IGKV4-1*01	IGKJ4*01	QQYYNTPLT	9	IGKC					
N6PMPB174	IGHV3-23*04	IGHD2-21*01	IGHJ6*04	DRGKERRPKQTGKSYYYYYGLDV	23	2	IGHA1	IGKV3-15*01	IGKJ2*02	QQYNNWPLYT	6	IGKC					
N6PMPB147k	IGHV4-4*07	IGHD3-10*01	IGHJ6*02	AGAVLTPLWFGETHYYYYPMDV	22	13	IGHG1	IGKV3-20*01	IGKJ2*01	QQYGTSHLYT	12	IGKC					
N6PMPB247	IGHV4-59*01	IGHD1-26*01	IGHJ4*02	GGGWDLLDY	9	19	IGHG2	IGKV1D-39*01	IGKJ3*01	QQSGTTPL	13	IGKC					
N6PMPB179	IGHV3-7*01	IGHD3-3*01	IGHJ4*02	APQYFDFWSSDY	12	2	IGHG1						IGLV3-1*01	IGLJ3*01	QAWDSSTVI	2	IGLC7
N6PMPB302	IGHV3-7*01	IGHD3-9*01	IGHJ4*02	GGQAWIQLWYFDY	13	10	IGHG1	IGKV1-6*01	IGKJ3*01	LQDYNYPFT	10	IGKC					
N6PMPB112	IGHV3-15*01	IGHD6-25*01	IGHJ6*02	GVGMGGMDV	9	6	IGHG1						IGLV9-49*01	IGLJ3*02	GADHGSGSNFFWV	2	IGLC7
N6PMPB124	IGHV3-9*01	IGHD3-10*01	IGHJ4*02	DRLKYYGSGSPLDY	14	8	IGHG1						IGLV3-21*01	IGLJ3*02	QVWHTSSDHRGV	8	IGLC2
N6PMPB325	IGHV3-72*01	IGHD2-21*02	IGHJ4*02	LSGSY	5	15	IGHM						IGLV1-47*01	IGLJ3*02	AVWDDSLSGRV		IGLC2
N6PMPB298	IGHV3-11*01	IGHD3-3*01	IGHJ6*02	DQAALKNFDFWSAYPAPQYYFG	25	26	IGHG4	IGKV3-15*01	IGKJ2*01	QHYNNWPYT	10	IGKC					
N6PMPB297	IGHV1-3*01	IGHD2-2*02	IGHJ4*02	GYCSSTSCQYYFDY	14	20		IGKV4-1*01	IGKJ2*01	QQYYSIPYT	13	IGKC					
N6PMPB250	IGHV3-11*01	IGHD3-16*02	IGHJ5*02	GRGSYLPPGLDH	12	22	IGHA2						IGLV3-21*02	IGLJ3*01	QVWDSNRQHPYMI	20	IGLC7
N6PMPB356	IGHV3-11*01	IGHD4-17*01	IGHJ6*02	GTYGDYRQVGMDV	13	23	IGHA2						IGLV1-47*01	IGLJ3*01	ATWDDSLGGL	16	
N6PMPB153	IGHV3-15*01	IGHD5-5*01	IGHJ4*02	VFLNDRLLGYTYGWSSVFDY	20	14	IGHG1	IGKV1D-39*01	IGKJ3*01	QQSFSSLGPT	24	IGKC					
N6PMPB365	IGHV3-23*04	IGHD5-24*01	IGHJ4*01	ALYNDRWFFDY	11	31	IGHA1	IGKV3D-11*01	IGKJ4*01	QQRGNWPLT	13	IGKC					
N6PMPB294	IGHV1-8*01	IGHD7-27*01	IGHJ1*01	AVHTNYMGQVLYSLDS	16	18		IGKV1-39*01	IGKJ4*01	QQSYLTPFT	18	IGKC					

Table 30: Legend for Vax2 Igs for fig. 10

Ig ID	Ig Name	Ig ID	Ig Name
B21	N5PMPB045	B01	N5PMPB025
E16	N5PMPB112	F11	N5PMPB131
B05	N5PMPB029	A13	N5PMPB013
D13	N5PMPB085	E22	N5PMPB118
A23	N5PMPB023	G12K	N5PMPB156_K
F14	N5PMPB134	F16	N5PMPB136
G20	N5PMPB164	F07	N5PMPB127
E15	N5PMPB111	A14	N5PMPB014
C14	N5PMPB062	A12	N5PMPB012
D02	N5PMPB074	D09	N5PMPB081
A03K	N5PMPB003	D16	N5PMPB088
G09	N5PMPB153	A03K	N5PMPB003
C08	N5PMPB056	B13	N5PMPB037

Table 31: Legend for Vax3 Igs for fig. 10

Ig ID	Ig Name	Ig ID	Ig Name
P11	N6PMPB371	O20	N6PMPB356
D19L	N6PMPB091_L	K10	N6PMPB250
D19K	N6PMPB091_K	M09	N6PMPB297
P11	N6PMPB371	N06	N6PMPB318
I06	N6PMPB198	O14	N6PMPB350
E09	N6PMPB105	O10L	N6PMPB346_L
M15	N6PMPB303	P01	N6PMPB361
H13	N6PMPB181	O19	N6PMPB355
G03K	N6PMPB147_K	A08	N6PMPB008
H11	N6PMPB179	P17	N6PMPB377
M14	N6PMPB302	P13	N6PMPB373
F04	N6PMPB124	G17	N6PMPB161
N13	N6PMPB325	G04	N6PMPB148
M09	N6PMPB297	F15	N6PMPB135
K10	N6PMPB250	O09	N6PMPB345
G09	N6PMPB153	O13	N6PMPB349
M06	N6PMPB294	A15	N6PMPB015
E18	N6PMPB114	H09	N6PMPB177
F18	N6PMPB138	F14	N6PMPB134
L03	N6PMPB267	G03L	N6PMPB147_L
H22	N6PMPB190	L15	N6PMPB279
J08	N6PMPB224	E17	N6PMPB113
P24	N6PMPB384	H06	N6PMPB174
I22	N6PMPB214	O08	N6PMPB344
O09	N6PMPB345	N09	N6PMPB321
N06	N6PMPB318	E16	N6PMPB112
M10	N6PMPB298	O03	N6PMPB344
L13	N6PMPB277	O20	N6PMPB356
M10	N6PMPB298	P05	N6PMPB365
E04	N6PMPB100	K07	N6PMPB247
O10K	N6PMPB346_K		

Table 32: Legend for Vax4 Igs for fig. 10

Ig ID	Ig Name	Ig ID	Ig Name
K22	N7PMPB262	K21	N7PMPB261
J14	N7PMPB254	J12	N7PMPB228
K05	N7PMPB245	K19	N7PMPB259
H14	N7PMPB182	K18	N7PMPB258
J19L	N7PMPB235	K01	N7PMPB241
K02	N7PMPB242	L12	N7PMPB276
I04L	N7PMPB196	I14	N7PMPB206
K15K	N7PMPB255	J20	N7PMPB236
K02	N7PMPB242	L17K	N7PMPB281
J19	N7PMPB235		